

**WILDLIFE SURVEILLANCE SYSTEMS-
CHRONIC WASTING DISEASE**

**A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Department of Large Animal Clinical Sciences
Western College of Veterinary Medicine
University of Saskatchewan
Saskatoon**

**By
Joanne R. Tataryn BSc, DVM**

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ABSTRACT

Increased demand for animal disease surveillance information has led to the development and refinement of methodologies for qualitative and quantitative surveillance system evaluations to maximize efficiency and efficaciousness. The impetus for this surveillance evaluation project was chronic wasting disease (CWD) and the objectives were to apply both qualitative and quantitative methodologies to examine the components of CWD surveillance in Saskatchewan.

A retrospective review of deer pathology and hunter-harvest submissions in Saskatchewan was conducted through the Canadian Cooperative Wildlife Health Centre. Qualitative evaluation methods outlined by Klauke et al (1988) were used and included key stakeholder interviews. A quantitative evaluation, with specific focus on disease detection, was conducted to examine system sensitivity, confidence of disease freedom and to compare system components using methods described by Martin et al (2007). The analysis was conducted using a scenario tree and Monte Carlo simulation.

Sampling rates of dead and clinically ill deer were low with a high degree of variability by season, year, location and nature of submissions. Ultimately, variability of submission patterns likely affected when and where diseases were detected. Poor data quality reduced the amount of available data for analysis but quality dramatically improved over time.

The surveillance evaluation demonstrated that the current surveillance system places more emphasis on monitoring trends in CWD-positive areas, at the expense of early detection. This is explained mostly by the coupling of disease control efforts and surveillance, in that harvests are heavily focused in CWD-

positive areas. The system is not sufficient to detect disease in new areas where the disease prevalence is low, primarily due to low submission rates.

The quantitative evaluation found that overall sensitivity of the surveillance system and confidence of disease freedom was highly dependent on detection prevalence and the ongoing risk of disease introduction. Surveillance in the eastern part of Saskatchewan was not adequate from 1997-2006 to detect CWD at 0.5-1% prevalence. However, if risk of CWD introduction over this time period was assumed to be low, it can be concluded that the prevalence in this region was not 5% or higher.

A detection goal of 0.5-1% prevalence is an ambitious surveillance goal, especially in areas where the risk of disease introduction is high. The use of more targeted surveillance strategies should be further explored to help better meet surveillance these surveillance objectives.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my co-supervisors, Drs. John Campbell and Trent Bollinger, whose knowledge, vision and encouragement during project development and implementation made for some interesting times and some great learning and growing. I would also like to thank the other members of my committee, Drs. Ted Leighton, Tasha Epp and John Berezowski (external examiner) for their valuable contributions and insights.

Many thanks to Marnie Zimmer, Alexia Reid, and crew with the Canadian Cooperative Wildlife Health Center (CCWHC) for their patience and assistance with data abstraction and management. I would also like to thank Tracy Creighton for her GIS expertise and map generation.

This project would not have been possible without the financial assistance of the Interprovincial Graduate Student Fellowship Program, Western College of Veterinary Medicine and the University of Saskatchewan. Additionally, financial support for a portion on my tuition was extended through the Canadian Food Inspection Agency, which I'm grateful for.

I would also like to especially thank my lovely husband (Anthony) and two boys (Luke and Ben) for putting up with me during this whole process and continuing to encourage and support me to the end.

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LIST OF ABBREVIATIONS

3D- Dead, diseased, dying
BSE- Bovine Spongiform Encephalopathy
CCWHC- Canadian Cooperative Wildlife Health Centre
CDC- Center for Disease Control, United States
CDMS- Cooperative Deer Management Survey
CFIA- Canadian Food Inspection Agency
CNCWDS- Canadian National Chronic Wasting Disease Strategy
CNWDS- Canadian National Wildlife Disease Strategy
CWD- Chronic wasting disease
EHD- Epizootic hemorrhagic disease
ELISA- Enzyme-linked immunosorbent assays
FAO- Food and Agriculture Organization of the United Nations
HACCP- Hazard Analysis Critical Control Point
HRA- Herd reduction area
IHC- Immunohistochemistry
NWD- National Wildlife Disease database
OIE- World Organization for Animal Health
PDS- Prairie Diagnostic Services
PrP^C- Normal cellular glycoprotein
PrP^{CWD}- Abnormally refolded prion protein, pertaining to CWD
PrP^{SC}- Abnormally refolded prion protein, pertaining to scrapie
P*_H- Herd design prevalence, probability that a herd is infected
P*_U- Animal design prevalence, probability that an animal is infected, given the herd is infected
RLN- Retropharyngeal lymph node
SMOE- Saskatchewan Ministry of the Environment
SPS- Sanitary and Phytosanitary agreement
SS- Surveillance system
SSc- Surveillance system component
TSE- Transmissible spongiform encephalopathy
USA- United States of America
UTM- Universal transverse mercator
WCVm- Western College of Veterinary Medicine
WHO- World Health Organization
WMZ- Wildlife management zone
WTD – White-tailed deer (*Odocoileus virginianus*)

1. INTRODUCTION

The demand for animal disease surveillance information has increased in recent years with the emergence and re-emergence of new and old infectious diseases affecting both humans and animals alike. Diseases such as influenza (avian and swine variants) and West Nile virus have both necessitated and enabled animal and human health jurisdictions to work together to meet these new challenges. Surveillance systems are at the core of animal and public health management policy decisions and this increased demand has contributed to the development and refinement of methodologies for qualitative and quantitative surveillance system evaluation. Surveillance evaluations, regardless of methodology are currently under-utilized and more regular, systematic evaluations would help to improve efficiency, efficacy and appropriate resource allocation. The impetus for this surveillance evaluation is chronic wasting disease, which has continued to spread throughout Saskatchewan in recent years despite aggressive disease control efforts and which poses a unique and increasing surveillance challenge.

In Saskatchewan, CWD surveillance in wild cervids is used to identify infected populations, monitor trends, and measure the effectiveness of disease management efforts. The program primarily relies on samples from healthy deer that are submitted by hunters; there is also limited testing of compromised, clinically diseased deer (diseased, dying or dead) through the Canadian Cooperative Wildlife Health Centre's wildlife diagnostic pathology service. Among these, is a subset of deer with clinical signs consistent with CWD. It is intuitive that targeting surveillance efforts toward CWD 'suspects' (poor body condition with neurologic dysfunction) would be an efficient way to detect disease; these animals have a greater probability of being infected. However it is difficult to quantify the benefits of targeted testing and to interpret negative findings using this strategy.

Surveillance evaluations can be used to help further describe the value of targeted testing and to assist with interpreting negative findings. Similar to the

sensitivity of a diagnostic test which estimates the confidence we may have in the test results, the sensitivity of a surveillance system can be calculated to quantify the probability that the disease will be detected by the surveillance system above a certain pre-set threshold. From this, we can describe negative surveillance results in terms of disease freedom; we can also compare surveillance systems in order to determine which system will work best to meet objectives.

1.1 Objectives

This project has the following objectives: 1) To retrospectively review white-tailed and mule deer diagnostic pathology submissions from 1966-2006 in Saskatchewan in order to characterize this passive surveillance system and describe submission patterns, including demographic, temporal or spatial trends 2) To describe the current CWD surveillance system 3) to conduct a qualitative surveillance evaluation and examine the use and value of diagnostic pathology submissions in the surveillance of chronic wasting disease as compared to hunter-harvested sampling techniques, 4) To model CWD surveillance systems using a scenario tree and 5) To conduct a quantitative evaluation using stochastic simulation modeling to calculate the sensitivity of the CWD surveillance system and quantify the value of target testing.

2. LITERATURE REVIEW

2.1 CHRONIC WASTING DISEASE

2.1.1 Agent

Chronic wasting disease (CWD) is a fatal, infectious, neurodegenerative prion disease (transmissible spongiform encephalopathy; TSE) that affects both free-ranging and captive cervid species in North America. Prions are devoid of nucleic acid and are composed of modified protein which causes normal cellular glycoprotein (PrP^C) to become abnormally refolded (PrP^{CWD}) (Prusiner, 1998).

2.1.2. Susceptible species

Species known to be naturally susceptible to CWD are mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus*) and moose (*Alces alces*) (Williams and Young, 1980; Williams and Young, 1982; Williams and Young, 1992; Kreeger et al., 2006). Black-tailed deer (*Odocoileus hemionus columbianus*), a subspecies of mule deer are also susceptible to CWD (Williams, 1980). Although there are many similarities between CWD outbreaks in deer and elk, there are some differences. Prevalence in wild elk is much lower than in wild deer. This could be partly attributed to the distribution and quantity of prion in different tissues throughout the body. PrP^{CWD} levels have been found to be lower in lymphoid tissues of elk compared to deer, which suggest that infected deer may be more likely than elk to transmit the disease (Race et al., 2007).

It is considered likely that other subspecies of white-tailed deer, mule deer, and elk would also be susceptible if sufficiently exposed (Williams, 2005).

Susceptibility of caribou (*Rangifer tarandus*) to CWD remains unknown, but some level of susceptibility seems likely based on similarities between the normal cell prion proteins of caribou and mule deer (Bollinger et al., 2004; Happ et al., 2007). This is of great concern as caribou typically have large herd sizes, seasonal aggregations and range fidelity; therefore there is a high potential for CWD to

spread in northern Canada, if it were to be introduced in this species (Bollinger et al., 2004). CWD has not been successfully transmitted by oral inoculation to species outside of the cervid family, suggesting a strong species barrier (Sigurdson, 2008).

Cattle appear to be highly resistant to CWD, and cattle did not become infected after years of co-grazing with CWD-infected mule deer, grazing in CWD-infected areas, or following direct oral exposure of mule deer CWD prions (Gould et al., 2003; Williams, 2005; Sigurdson, 2008).

Although there is no definitive evidence, sheep are more likely to be susceptible to CWD. They have been shown to be susceptible via intracerebral inoculation (Hamir et al., 2006). The susceptibility of sheep to CWD by oral exposure has not been studied (Williams, 2005).

2.1.3 Transmission

CWD is transmitted horizontally through direct contact with infected animals and indirectly by exposure to environments contaminated with PrP^{CWD} containing secretions (saliva), tissues or decomposed carcasses (Sigurdson, 2008; Miller and Williams, 2003; Miller et al., 2004; Mathiason et al., 2006). The role of maternal transmission appears to be minimal (Miller and Williams, 2003), although elk calves born to positive dams are at greater risk of CWD, likely due to increased lateral transmission via close association (Argue et al., 2007). A recent study examining whether infectious PrP^{CWD} in body fluids and excreta were capable of inducing CWD showed successful transmission to white-tailed deer fawns via saliva (50ml X 3 inoculations) given orally, and blood (250mL X 1 inoculation) given intravenously and intraperitoneally. Repeated exposures of urine (50mL) and feces (50g) given orally did not produce detectable levels of PrP^{CWD}, however small sample size, elective preclinical termination, and genetic variation in individual susceptibility may have resulted in type II error (Mathiason et al., 2006).

The point following exposure at which an animal become infectious is not known, however it is believed that shedding of PrP^{CWD} begins some time around the onset of clinical signs and is progressive throughout the course of the disease (Williams and Miller, 2002; Miller et al., 2000). The average survival time of an infectious clinically affected deer is estimated to be about 1.05yr (Miller et al., 2000).

The main factors which drive the geographic expansion of the disease are not currently known, however it has been speculated that juvenile dispersal movement and disease spread among matriarchal social groups may play a role (Joly et al., 2006).

2.1.4 Pathophysiology

Cervids are thought to become infected via the oral route. PrP^{CWD} is first identified in the tonsils and gut-associated lymphoid tissues (Williams, 2005). Sigurdson et al. (1999) found PrP^{CWD} accumulation in tonsils of mule deer as early as 78 days post-inoculation with CWD-positive brain material. PrP^{CWD} moves to the central nervous system at the vagal nucleus and thoracic spinal cord. This is followed by progressive involvement in the central and peripheral nervous tissue. Once the animal is terminally ill, a wide variety of tissues may be involved, including other lymphoid tissue, the endocrine system, spleen, heart (Fox et al., 2006; Sigurdson et. al, 2001) and skeletal muscle (Angers et. al., 2006).

Accumulation of the abnormal prion protein in the nervous tissue leads to destruction of normal nervous tissue, and results in the clinical signs observed. In deer, the incubation period is seldom less than 18 months (Williams and Young, 1993; Williams, 2005).

2.1.5 Clinical Signs

Clinical signs associated with CWD in wild cervids have not been well described. Clinical signs, as observed in captive animals, involve a progressive loss of body condition and neurologic signs. Behavioral changes, such as periods of lack of awareness, fixed stare, altered flight distances (increased or decreased), altered stance with lowered head, repetitive walking and hyperexcitability when handled may be noted (Williams, 2005). Other signs that may be observed include odontoprisis, excessive salivation due to difficulty swallowing, esophageal dilation and regurgitation causing aspiration pneumonia, polyuria/polydipsia and retention of winter hair coat (Williams, 2005; Argue et al., 2007). Deer with subclinical or early clinical infection are thought to be more susceptible to sudden death after handling than unaffected deer (Williams, 2005). Duration of clinical disease varies, however captive animals will typically show clinical signs for weeks to months. It is presumed that the duration may be somewhat shorter in free-ranging animals due to increased resource competition, and predator pressure (Williams, 2005). Those animals in clinical stages of infection are thought to be at increased risk for motor vehicle collisions (Krumm et al., 2005), hunter harvest (Conner et al. 2000), or being killed by predators (Williams, 2005).

Once an animal is infected, the disease is invariably fatal and there are currently no available treatments. There is no vaccine to prevent the disease.

2.1.6 Gross Pathology

Gross lesions are typically non-specific. Body condition may vary depending on the stage of disease. Animals that are either subclinical or in early stages of clinical disease, may be in good body condition. As the disease progresses, body condition tends to decline, with animals in terminal stages typically emaciated. Other indicators on gross pathology may be aspiration pneumonia, dilute urine, and rumen contents may be watery, or have increased amounts of sand or gravel (Williams, 2005).

2.1.7 Histopathology

Histologically, advanced disease is characterized by bilateral spongiform changes in the central nervous system with a noted lack of inflammatory response. Vacuolization occurs in the neuronal perikarya and neuronal processes (Williams and Young, 1980; Williams, 2005). Microscopic lesions are most obvious in the diencephalon, olfactory cortex, and nuclei of the medulla oblongata (dorsal vagal nucleus), however milder lesions are widespread in the brain and spinal cord (Spraker et al., 2002; Williams, 2005).

2.1.8 Disease detection

Abnormal prion protein (PrP^{CWD}) can be detected by immunohistochemistry (IHC) in a number of tissues. In addition to demonstrating PrP^{CWD} in nervous tissue, abnormal prion proteins are well distributed throughout the lymphoid tissue in deer and are readily detected by examining retropharyngeal lymph nodes and tonsillary tissue, often at very early stages of disease (Miller and Williams, 2002). A recent study (Hamir et al., 2006) demonstrated positive PrP^{CWD} by IHC in a preclinical white-tailed deer in the tonsils, spleen, various lymph nodes, Peyer's patches and ectopic lymphoid follicles in the renal hilus. Another study using rodent models of scrapie showed PrP^{SC} to be present in inflammatory lymphoid tissues in kidney, pancreas, and liver and showed that chronic inflammatory conditions may act as modifiers of prion transmission (Heinkenwalder et al., 2005). Accumulation of PrP^{CWD} in muscle tissue of infected animals is of concern if the animal is consumed. Angers et al. (2006) used skeletal muscle of CWD-infected mule deer and transmitted the disease to transgenic mice expressing cervid prion protein. PrP^{CWD} has been detected by IHC in cardiac myocytes of infected elk and white-tailed deer (Jewell et al., 2006) but the same study did not detect PrP^{CWD} in diaphragm, triceps brachii, semitendinosus, latissimus dorsi or tongue muscles. Although some studies using laboratory animals (Thomzig et al., 2003) have detected PrP^{SC} in skeletal muscle and tongue, other studies (Hamir et al., 2004; Jewell et al., 2006) have contradicted those findings, leaving the question regarding skeletal muscle infectivity still inconclusive. A recent study

demonstrated PrP^{SC} in skin of hamsters and sheep infected with scrapie; these findings suggest that in TSEs such as scrapie and CWD, virtually all tissues with nerve fibre innervations contain varying levels of prions during the clinical phase (Thomzig et al., 2007).

2.1.9 Disease diagnosis

Currently, testing in wild cervids in Saskatchewan involves using immunohistochemistry (IHC) techniques previously described (Spraker et al., 2002). Available postmortem tissues from tonsil, retropharyngeal lymph node and obex are tested for presence of the abnormal prion protein. In deer, testing lymphoid tissue will detect 99% of cases by IHC (Spraker et al., 2002); however in elk both brain and lymphoid tissue must be tested as approximately 10-15% of cases will have detectable levels of PrP^{CWD} in the brain, but not the lymphoid tissue (Spraker et al., 2004; Williams, 2005). A positive diagnosis is made when any one of the selected tissues mentioned above stain positive (Trent Bollinger, personal communication). Confirmatory testing is performed at the National Reference Laboratory for Transmissible Spongiform Encephalopathies, Canadian Food Infection Agency (Ottawa Laboratory, Fallowfield, Napean, ON, Canada) using immunohistochemistry.

Rapid test enzyme-linked immunosorbent assays (ELISAs) are also used, and have good sensitivity when compared with IHC (Hibler et al., 2003). Current live animal testing techniques, such as tonsillary biopsies, are available but logistics and cost preclude routine use and these are primarily used in research settings (Wild et al., 2002). Other live animal testing techniques currently being developed with some promise include rectal mucosa biopsies (Spraker et al., 2009; Spraker et al., 2006).

2.1.10 Implications of endemic CWD in wild cervid populations

Once a population is affected, it appears as though a protracted epidemic occurs, resulting in a steady increase in prevalence over time and space (Miller et al.,

2000). Long term population effects are unknown, however modeling done by Miller et al. (2000) suggests that 'epidemics sustained over 30 to 50 yr could reduce affected populations dramatically', and that epidemics would be 'self-limiting, driving the population to extinction'. This has been shown in captive situations where more intensive transmission occurs under confinement (Williams and Young, 1992). Because free-ranging populations occupy a larger geographic region, with more complex interactions, the ultimate outcome is unknown.

In Saskatchewan, CWD appears to be currently restricted to four distinct geographic regions (Figure 1). Survey results conducted since 2000, suggest that the local prevalence is increasing in some areas, regardless of disease control actions taken (T. Bollinger, personal communication).

In terms of impact on the population, the long-term effects of CWD may alter the genetic composition of herds affected. Species specific polymorphisms at the *Prnp* gene appear to affect susceptibility to CWD infection. It appears as though certain genetic lines are predisposed to shorter incubation periods of disease, while others experience a longer, more protracted course of disease. O'Rourke et al. (1999) was able to demonstrate that elk that were homozygous for methionine at codon 132 (132MM) were overrepresented among elk infected with CWD, when compared to elk heterozygous for methionine and leucine (132ML) or homozygous for leucine (132LL); experiments suggest that 132MM or 132ML elk develop clinical disease earlier (23-40 months) than elk with 132LL (59-64 months) (Sigurdson, 2008). Other studies have demonstrated that mule deer heterozygous for serine/phenylalanine at codon 225 (225SF) appear to have prolonged incubation periods, when compared with homozygous (225SS) deer (Jewell, et al., 2005; Fox et al., 2006; Sigurdson, 2008). White-tailed deer have a polymorphism at codon 96, and deer that express 96SS had a lower risk of CWD, but were not resistant (Sigurdson, 2008). Although this area needs further research to better understand the impact of genetic composition and disease

dynamics, it is conceivable that the genetic structure of populations could change over time and the impacts of this change on biodiversity is unknown.

There is a great economic cost associated with this disease through both disease surveillance management activities, and loss of hunting revenues. Management programs based on large herd reductions in affected areas may result in reduction of viewing opportunities and associated revenues (Bollinger et al., 2004) and are not well received by the public. Many landowners are concerned that the herd reductions disrupt their livestock and are reluctant to allow hunter access. Although initial observations in Canada and the USA indicate that the majority of hunters continue to hunt in areas even when CWD has been detected, there is concern that if CWD prevalence becomes high, public attitudes may change and human health concerns may reduce hunting activity (Bollinger et al., 2004).

Chronic wasting disease has also had a large impact on the Canadian game-farming industry. The discovery of chronic wasting disease in game-farmed populations in Canada led Korea to impose trade restrictions in December 2000 (Kim et al., 2005), contributing to a decline in both demand and price of Canadian elk velvet antler product (Kahn et al., 2004). Current farming practices do not appear to prevent disease transmission 'across the fence', and therefore the status of surrounding wildlife populations ultimately impacts the international perceived risks associated with Canadian cervids and their products.

There is currently no evidence to suggest that humans are susceptible to CWD (Anderson et al., 2007; Belay et al., 2001; Belay et al., 2003; Belay et al., 2004; Davis et al., 2003; MaWhinney et al., 2006). However, as with Bovine Spongiform Encephalopathy (BSE), it is possible that humans are highly resistant to CWD infection and without extensive, prolonged exposure to high doses of prions, as occurred in the United Kingdom with BSE, it would be very difficult to prove susceptibility. In addition, it is unclear what the clinical presentation and

incubation period may be; leaving it difficult to prove conclusively that humans are not susceptible. And so, the precautionary principle is used regarding CWD's potential risk to humans. It is advised that hunters not harvest animals that appear ill, and that they wear protective gear when dressing carcasses, avoiding contact with high risk tissues (brain, lymph tissue and spinal cord). It is also recommended that tissues from CWD-infected deer or elk are not used for animal or human food (WHO, 2002).

2.1.11 Risk factors associated with chronic wasting disease

a) Sex

Studies of the sex distribution of CWD are limited as few regions harvest enough females to do good comparisons. Miller et al. (2000) did not find a large difference in prevalence between sexes in any of the three species studied. However, there appeared to be sex-specific differences between broad age classes of mule deer (≤ 3 year old, ≥ 4 year old). This could be partly explained by disparity in underlying age structures from local hunting pressure on males (Miller et al., 2000). They did not show differences in infection proportions between male and female white-tailed deer and elk.

b) Age

Transmission appears to occur at any age via lateral transmission, and research supports adult cervids can contracting CWD (Miller et al., 1998; Miller et al., 2000). Transmission can also occur at a very young age, but only rarely are yearlings clinically affected due to the extended incubation period (Williams, 2005). Current testing strategies, when applied to animals younger than 1 year of age do not typically show a high prevalence in this population (Joly et al., 2006). As a result, only animals greater than 18 months of age are typically tested for CWD (Schettler et. al., 2006).

c) Spatial clustering

Miller et al. (2000) found prevalence varied with spatial region, and tended to follow biologically relevant spatial patterns (lower elevation foothill subpopulations tended to be most severely affected). Joly et al. (2006) also found CWD to have a clustered spatial distribution in deer populations in south-central Wisconsin. Spatial analysis for clustering identified a core-area of higher prevalence with a decline in prevalence from the center of the affected area; findings consistent with other horizontally transmitted diseases (Hickling, 2002; Joly et al, 2006). Joly et al (2006) hypothesized that the prevalence of CWD at any particular point is correlated with distance from introduction (as a surrogate for time) and local habitat conditions. Joly et al. (2006) also found that prevalence was positively correlated with the amount of deer habitat available.

d) Density

The role of density in CWD transmission is unknown. It is believed that animal-animal transmission is driven primarily by the average number of contacts per infectious individual per year, independent of density (Heesterbeek and Roberts, 1995; McCarty and Miller, 1998; Miller et al., 2000). This is further supported by the fact that population reduction efforts, aimed at reducing density and reducing transmission, have not been successful in eliminating the disease (Miller et al., 2000). However, it is foreseeable that cervid populations at higher densities, including artificial situations such as feeding sites and captive populations, would have increased contacts per year, hence increased transmission rates. A more recent study comparing prevalence rates from wild and captive cervid populations suggest that CWD transmission may be facilitated at higher cervid densities (Joly et al., 2006) which would act to increase the local prevalence of the disease.

e) Seasonality

Affected animals are more commonly reported in the fall and winter (Williams, 2005). It is unknown if this factor is associated with observational bias or with

increased environmental stress on already compromised CWD infected individuals.

2.1.12 Geographic distribution

Chronic wasting disease is not known to exist in wild cervid populations outside of North America, although surveillance in many countries is limited (Schettler et al., 2006; Roels et al., 2005). Canadian CWD-infected game-farmed elk were imported by Korea however to date, no Korean-born deer have been reported with CWD (Kim et al., 2005). The current geographic distribution of known CWD-positive free-ranging and game farmed cervids as of January 2009, is given in Figure 2.

2.1.13 Origins of CWD

CWD was first identified in Colorado in captive cervids as early as 1967 (Williams and Young, 1980). It was initially thought to be limited to captive wildlife facilities, however it was later discovered in free-ranging cervids in 1981 (Spraker, 1997). The source of the initial cases of CWD will never been known; the primary hypotheses include spontaneous generation, and mutation from other TSEs, such as scrapie (Kahn et al., 2004).

2.1.14 History of CWD in Canada

Since CWD was first identified in Colorado in the 1960s, the continent has watched the apparent geographic spread of this disease. CWD was first identified in Canada in 1980 at the Toronto zoo. This captive mule deer died in 1978 after exhibiting signs consistent with CWD. Retrospective investigation identified an additional 7 mule deer and 1 black-tailed deer infected with CWD (Dubé et al., 2006). The most plausible source of introduction was the importation of infected animals from Colorado, although this has not been established conclusively (Dubé et al., 2006). No further cases of CWD were identified in Canada until 1996, when a game-farmed elk from Saskatchewan was presented for necropsy with a history of chronic weight loss and was

subsequently diagnosed with CWD. Another positive game-farmed elk was identified in 1998, and in 2000, a third case was diagnosed which led to identification of what is believed to be the source farm (index farm) responsible for transmission, either directly or indirectly, to a number of other farms. In 2000, an eradication campaign was undertaken which led to the destruction of over 8000 animals on 40 farms. Epidemiologic investigation indicated that CWD was likely introduced into the farmed elk industry through importation of infected farmed elk from South Dakota in the late 1980s (Kahn et al., 2004).

In response to the discovery of infected game-farmed elk, Saskatchewan Ministry of Environment (SMOE) conducted a survey in wild deer in the area surrounding the infected farm, all with negative results (CCWHC, 1998). Experts at the time were hopeful that the disease was not present in the wild and that the outbreak had been adequately contained. It was estimated that the likelihood of infection being present in wild cervids was very low (CCWHC, 1996). However, the first case of CWD in a wild mule deer was identified in 2000 from the Manitou Hills region of western Saskatchewan near Lloydminster, close to the Alberta-Saskatchewan border (CCWHC, 2001). This has led to speculation that CWD in wild cervid populations is a result of a 'spillover' from farmed cervids. Other possible sources of infection include sporadic occurrence, spillover from scrapie infected animals, and natural migration from southern infected populations (Kahn et al., 2004; Bollinger et al., 2004).

2.1.15 Current Surveillance strategies for CWD

Current CWD surveillance programs in wild cervids are based primarily on testing hunter-harvested animals. The advantage of this methodology is that samples can be collected and obtained at minimal cost. The samples are usually biased towards regions classified as higher risk (those regions known to be infected, adjacent to known infected wild populations, or regions adjacent to known positive game farms). Samples within regions are not random, as more mature males are usually harvested, and animals infected with CWD may be

overrepresented due to increased susceptibility to harvest (Conner et al., 2000; Williams, 2005).

Another surveillance strategy used by some US wildlife jurisdictions is targeting and testing suspect animals, or “higher-risk” animals. This is typically done either through passive surveillance, relying on individuals to report any animals observed to be acting abnormally, or through a more active means, by collecting dead deer, including road kill and winter mortalities. One great advantage of targeted surveillance is that CWD-suspects have an increased probability of having the disease. One of the greatest limitations of this method is that this bias towards high risk animals makes interpretation of negative results difficult. A study conducted by Miller et al. (2000) compared testing of “suspect” deer and elk to hunter-survey deer and elk in CWD-endemic regions in Colorado from 1978-1999. A deer or elk was considered a clinical suspect if they were ‘showing clinical signs consistent with CWD (=1.5 yr, poor body condition, abnormal behavior, ± other signs)’ (Miller et al. 2000). “Clinically Suspect” deer were 13 times more likely, and “clinically suspect” elk were 86 times more likely to be infected with CWD in areas with endemic CWD, when compared to deer harvested by hunters (Miller et al., 2000). The caveat was that local prevalence in a region needed to reach 1% before clinical cases were detected. There was no correlation between number of clinically suspect CWD cases detected and CWD prevalence (Miller et al., 2000).

2.1.16 Current Management strategies for CWD

Currently, there is no treatment or vaccine for CWD and disease control has been attempted by wildlife authorities largely through both movement restrictions to prevent new foci of disease, and heavy culling in affected areas. The primary goal of these culling programs is to reduce the prevalence of CWD or, at best, eliminate the disease in a geographic region. The assumption underlying this strategy is that transmission is density-dependant and that population reduction will reduce contacts between susceptible and infected individuals, and thus

reduce transmission (Joly et al, 2006). There is some evidence that density plays a role in transmission by influencing the frequency and intensity of interactions among deer within or among social groups (Joly et al, 2006; Hirth, 1977; Nixon et al, 1991; Grenier et al., 1999; Kie and Bowyer, 1999). However, factors such as breeding behavior, social interactions, movement patterns and practices that artificially concentrate deer result in a nonlinear relationship between density and contact rate, making it unlikely that population reduction would result in a linear reduction of disease transmission (Joly et al., 2006). Environmental persistence complicates efforts and act as a source of new infections, even when herd numbers are reduced drastically (Miller et al., 2004).

Overall, efforts to date have been largely unsuccessful in eliminating the disease and controlling spread in wild populations, and new strategies need to be developed. Further information on deer behavior, dispersal patterns and disease transmission may provide insight into better recommendations for management of this disease.

2.2. SURVEILLANCE FOR EMERGING WILDLIFE DISEASES

2.2.1 Surveillance – definitions

The term “surveillance” was first used during the French Revolution, when it meant “to keep watch over a group of persons thought to be subversive.” This term has been extended to animal health programs to define the process of watching over an animal population to determine whether a specific disease or a group of diseases makes an incursion. It attempts to determine presence/absence of a disease and if a disease is present, changes in prevalence and the rate and direction of spread over time (Salman, 2003).

Surveillance is ongoing, and includes observation, data collection, data analysis, and communication of results. There are three components of disease surveillance systems:

- 1) a defined disease monitoring system,

- 2) a defined threshold of disease at which an intervention should take place,
- 3) a defined set of interventions that will be undertaken when the threshold is reached (Salman, 2003).

'A reliable surveillance system is the key to early warning of a change in the health status of any animal population. It is also essential in providing evidence about the absence of diseases or in determining the extent of a disease which is known to be present' (Salman, 2003). Several types of surveillance methods exist, and are classified according to their function and data collection method (Salman, 2003). The methods chosen depend on the objectives of the surveillance program.

2.2.2 Surveillance techniques

Ultimately, the only way to know whether a disease exists in a population is to simultaneously test 100% of the population with a perfect test (100% sensitivity, 100% specificity). This would only represent the disease status of the population at the time of testing, and would not account for the risk of introduction after that time. Testing of the entire population would need to be repeated on a continuous basis to provide up-to-date prevalence calculations, accounting for risk of introduction (Martin et al., 2007). For the large majority of cases this is not practical or possible and we are left with testing subsets of the animal population of interest which are used to make inferences about the population as a whole.

The subset selected for testing largely depends on the goal of the survey. One method is simply to define the population, take a random sample and test those individuals. There are other techniques that randomly choose herds and test a number of individual animals within those herds. These methods are primarily done as surveys and have great surveillance value, especially when estimating prevalence in populations where the disease is known to exist.

a) Passive and active surveillance

Passive surveillance is defined as a fixed, routine method which relies on veterinarians, meat inspectors, farmers and others to report suspicious cases at their discretion. Examples include submissions to a diagnostic pathology laboratory and reportable disease programs (Racloz, 2007). In the case of wildlife, conservation officers, hunters and members of the public comprise the front-line in disease detection. Despite their name, passive surveillance systems require a significant amount of effort to find ways and means of collecting and analyzing valuable information that may come out of these systems. They have the advantage of being relatively inexpensive, when compared to active surveillance systems, because the information is already being collected for some other reason. The greatest disadvantage of passive surveillance systems is that often the samples being collected are not representative of the entire population and information about the population from which the samples originated is often lacking. As a result, one must be cautious in interpreting the results at a population level.

Passive systems are often used as the first step in identifying new and emerging diseases. When a new disease is introduced into a population, time to detection depends on the severity of clinical signs (morbidity and mortality), the familiarity of the individuals (conservation officers, hunters etc) with the clinical signs of the disease, and the speed with which the disease spreads (Salman, 2003).

Stigmatized diseases, mild disease, diseases with non-specific clinical symptoms, long incubation periods, low within-herd and between-herd spread, and those diseases with unfavorable cost-benefit ratios, often have low case-reporting levels; and thus are difficult to assess using passive surveillance systems alone. Doherr et al (2001) reported an increase in case-ascertainment and reporting of BSE suspect cases in Switzerland by the passive system after renewing awareness through continuing education workshops for practitioners and owners and by implementing targeted BSE screening at dead yards and changing the whole-herd culling policy in BSE positive herds. They believed that

without a targeted surveillance program, mandatory clinical suspect reporting will only capture <50% of the detectable cases. In other TSE diseases such as for scrapie in sheep and goats, underestimation levels between 30 and 87% have been reported (Schrüeder et al., 1993; Hoinville et al., 1999; Baumgarten et al., 2000).

Active surveillance involves the purposeful collection of information, often targeting a specific disease. Surveys, sentinel systems and mass screening methods are examples of active surveillance (Racloz, 2007). This process produces more accurate estimates of frequency of disease such as incidence and prevalence, however they are often more expensive to implement.

b) Probability and non-probability sampling

Surveillance can also be categorized by the way the observation units are chosen: probability sampling (random) or non-probability sampling (non-random). Examples of non-random sampling include targeted and sentinel surveillance. For a disease such as BSE, which in Canada is rare, detection in the general population would require very large numbers of cattle to be tested in order to detect the disease. Targeted sampling of the bovine subpopulation considered at higher risk (4Ds- down, diseased, dying or dead) significantly improves the power to detect the disease.

c) Risk-based surveillance

The premise behind risk-based surveillance is: issues of higher risk are higher priorities for surveillance resources and as investments, also yield higher benefit-cost ratios (Stärk et al., 2006). 'In risk-based designs, public health, economic and trade consequences of diseases play an important role in selection of diseases or hazards. Furthermore, certain strata of the population of interest have a higher probability of being sampled for detection of diseases or hazards' (Stärk et al., 2006). The goal of using risk-based surveillance is to maintain

efficacy of traditional systems, but improving their efficiency, thus better using resources.

2.2.3 The importance of disease surveillance of wildlife

Changing patterns of human behavior, such as urban development, production intensification and increased movement of biologic life, has accelerated and intensified emergence and spread of infectious diseases. Wildlife can serve as either the originating source, or the reservoir for significant diseases which impact human, domestic livestock or naïve wildlife populations. As a result, organizations such as the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (OIE) have recognized the increased importance of wildlife health surveillance (FAO/WHO/OIE, 2004) and provided endorsement for enhancing wildlife monitoring and surveillance programs.

a) Disease surveillance of wildlife to protect our ecosystem

Disease is a normal part of a balanced ecosystem. However, human manipulation of ecosystems through activities such as habitat fragmentation, poaching, monoculture, and translocations has resulted in situations of imbalance which has fostered the emergence of new diseases or provided opportunity for diseases in situations where there weren't previously. Wildlife populations can be used as indicators of ecosystem health, much akin to the 'canary in the coal mine', and provide early warning of impending environmental disaster.

b) Disease surveillance of wildlife to protect public health

Forty years after the 'war on infectious disease' was declared by top scientists to be over, emergence of new diseases such as acquired immunodeficiency syndrome (AIDS) and re-emergence of diseases such as tuberculosis left researchers bewildered. After such a drastic decline in infectious disease mortality since the early 1900s, no one would have predicted a 58% increase in

infectious disease mortality in the US from 1980-1992 (Pinner et al., 1996). Of the infectious diseases considered to be emerging, 75% are zoonotic (Taylor et al., 2001) and wildlife populations provide a murky 'zoonotic pool' from which pathogens may emerge' (Morse, 1995). Diseases such as AIDS, SARS, plague, Nipah virus, West Nile virus, and Hendra virus are just a few examples of diseases thought to originate in wildlife populations (Jones et al., 2008).

c) Disease surveillance of wildlife to protect livestock populations and trade

There are many diseases known to infect both domestic and free-ranging populations, and many examples of spillover and spillback between the two compartments. Therefore, it is inevitable that diseases of importance to domestic animal health and trade will also have significance in wildlife populations. With the development of the Sanitary and Phytosanitary (SPS) agreement of the World Trade Organization (WTO) during the 1990s, countries are now required to provide scientific evidence documenting their disease status in order to improve fairness and transparency. A country can no longer claim disease-free status with a substandard disease detection system in place, and scientific evidence needs to be presented to substantiate disease freedom claims. In situations with an extended livestock-wildlife interface, disease-free claims may also include assessing high-risk free-ranging populations. If a free-ranging population is found to be infected with a listed OIE reportable disease, in absence of evidence of the disease in domestic populations, evidence must be put forth to substantiate compartmentalization of these two sectors to avoid trade restrictions.

2.2.4 Wildlife disease surveillance – The Canadian Approach

In response to increased national and international obligations to manage wildlife disease, the Canadian Cooperative Wildlife Health Center was formed in 1992 in Canada, to support wildlife disease surveillance, research and response. These activities were further enhanced with the development of a national policy framework in 2004: the Canadian National Wildlife Disease Strategy (CNWDS).

This strategy aims to ‘minimize the negative impacts of wild animal diseases on biodiversity, human and livestock health, the environment and the economy’ (CNWDS, 2004). The six primary goals of managing wildlife disease in Canada, as outlined by the strategy are: 1) prevention, 2) early detection, 3) rapid response, 4) effective disease management, 5) education and training, and 6) communication.

Monitoring disease in wildlife populations can be very challenging and expensive. Traditionally, passive surveillance systems or opportunistic sampling have been utilized as the keyhole through which we view wild animal health and disease. There are great advantages to such systems; they are relatively inexpensive, and coupled with education and awareness campaigns, they can yield good results (eg. West Nile virus dead wild bird surveillance program). Increased demand for wildlife disease information has placed increased pressure on these systems to produce reliable information, often requiring enhancements to existing systems. Like other veterinary disciplines, and perhaps even to a greater extent, wildlife health and management is further challenged with limited resources; hence the need for strategic risk-based surveillance plans to ensure efficient use of resources.

2.2.5 Negative surveillance findings and probability of disease freedom

In recent times, increased international trade between countries has resulted in increased demand for science-based assessments of a country's disease status. In 1995, member countries of the World Trade Organization (WTO) developed the SPS agreement (<http://www.wto.org/>) which requires countries to substantiate claims of disease freedom with proof. Countries are not allowed to institute trade barriers without having scientific evidence that their population would be at increased risk should the trade in question be allowed. As mentioned previously, guaranteeing all animals in a population to be free of disease would require continuous census sampling with a perfect test; neither practical nor possible in most situations.

It has been accepted that negative findings in adequate numbers of representative animals (adjusting for imperfect test performance), is sufficient to claim 'disease freedom' with some confidence (that if the disease is present, it is below an acceptable threshold) (Martin et al., 2007). Disease freedom claims have historically been substantiated primarily through two methods; 1) qualitative reviews by expert panels of complex surveillance systems (including non-representative samples, such as abattoir and targeted sampling methods), and 2) quantitative analyses based on structured, representative surveys (Martin et al, 2007). Both have their disadvantages; qualitative reviews, although rigorous are difficult to repeat and may not be transparent, while structured surveys are often expensive, difficult to implement, and reliance solely on survey results ignores the potential value of other sources of evidence (Martin et al., 2007). Recently, Martin et al. (2007) proposed a quantitative approach by which multiple surveillance sources can be analyzed and integrated to develop a quantitative probability estimate to support claims of freedom of infection in a population, thus allowing better utilization of information obtained from non-representative surveillance systems.

2.2.6 Evaluation of surveillance systems

International standards for conducting surveillance provides guidance for decision making by animal health authorities and allows for comparison and evaluation of surveillance systems. Currently, there are standards set for only a few selected diseases such as rinderpest and contagious bovine pleuropneumonia (Anon., 1997; Anon., 1998). There are no surveillance standards for chronic wasting disease. In the absence of surveillance standards, any surveillance system can be profiled by: objectivity, target population(s), design (sampling scheme and organization), diagnostic methods, analysis, and communication and feedback of results (Morris, 1991; Dufour and Audige, 1997; Dufour 1999; Stark, 2002). Once a surveillance system has been profiled and described, there are methods, qualitative and quantitative, which have been

proposed to further characterize the surveillance system in terms of quality. Regardless of methodology chosen, the approach must be objective, transparent and systematic (Salman, 2003). Hueston (1993) described an “ideal” surveillance system and then proposed that current systems could be compared with the “ideal” and scored accordingly. Dufour (1999) proposed using a similar scoring system, however, a more HACCP-like approach was taken, in which critical control points are identified and scored. These techniques have been criticized, largely for their susceptibility to subjectivity. A more quantitative approach was taken first by Baldock (2000) and Hueston and Yoe (2000) which used scenario trees and stochastic simulation to describe the pathways of disease detection and to determine the probability of detecting an infected animal (Stark et al., 2002). This technique has been further developed by Martin et. al (2007) allowing for multiple surveillance system components to be combined over multiple time periods to determine overall sensitivity. In order to gain the advantages of this method over more qualitative approaches there must be adequate, good quality data. Otherwise, the method is very similar to other approaches in subjectivity due to the use of expert opinion to fill in the missing data.

Surveillance systems should be systematically evaluated to ensure the objectives of the program are being met as efficiently as possible. Especially in situations where resources are limited, a critical evaluation of a surveillance system can be very advantageous to ensure good use of resources. Possible motivations for doing quality assessments include designing a new system, improving an existing one (quality or cost-effectiveness), deciding on acceptance of data produced, and determining equivalence in the context of international trade (Salman, 2003). Detailed protocols for conducting evaluations of surveillance systems have been prepared by the CDC and Klaucke, et al. (1988).

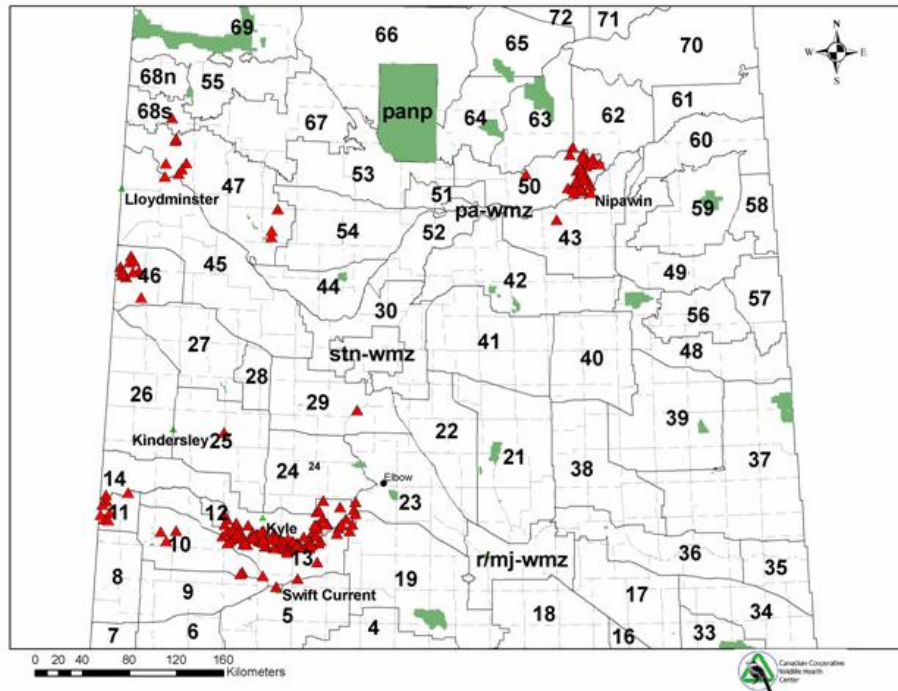
Despite their many uses, surveillance evaluations are highly underutilized in the veterinary field. It is the intent of the author to demonstrate the value of conducting surveillance evaluations in wildlife, and the different approaches to

evaluation will be explored in the context of CWD surveillance in free-ranging deer populations in Saskatchewan, Canada.

Figure 1 - Locations where chronic wasting disease has been detected in wild cervids (*Odocoileus hemionus*, *Odocoileus virginianus*, *Cervus elaphus*) in Saskatchewan

The Triangles Represent Locations of CWD Positive Wild Cervids in Saskatchewan, 2000-2008.

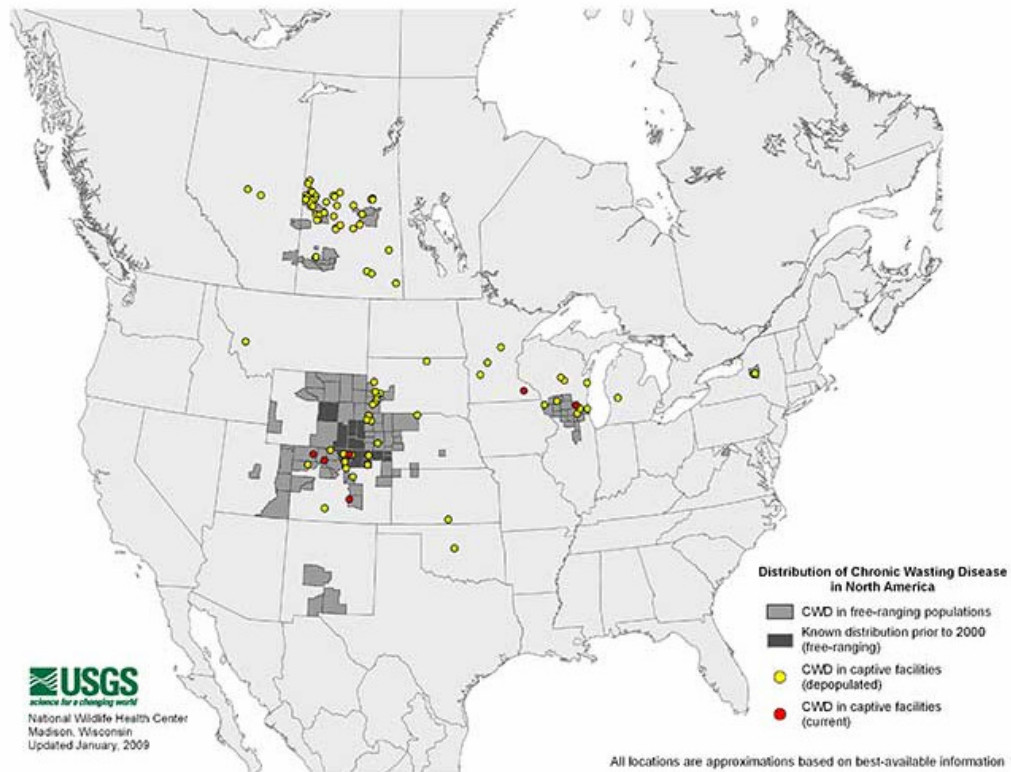
Numbered Areas are Wildlife Management Zones.



Source: http://wildlife1.usask.ca/en/cwd/chronic_wasting_disease.php

Figure 2 - Distribution of chronic wasting disease in free-ranging and captive cervid populations in North America

Source: http://www.nwhc.usgs.gov/disease_information/chronic_wasting_disease/index.jsp



Source: http://www.nwhc.usgs.gov/disease_information/chronic_wasting_disease/index.jsp

3. TEMPORAL, SPATIAL AND DEMOGRAPHIC PATTERNS OF FREE-RANGING DEER MORTALITY SUBMISSIONS TO A DIAGNOSTIC PATHOLOGY LABORATORY IN SASKATCHEWAN FROM 1966-2006

3.1 ABSTRACT

A retrospective study of free-ranging white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) submissions to a wildlife diagnostic pathology laboratory* from 1966-2006 was conducted to profile submission patterns in order to describe potential biases and to evaluate its use as a passive surveillance system to detect emerging diseases such as chronic wasting disease in wild cervids in Saskatchewan. Samples were characterized by species, year, type and nature of submission, age, sex, month submitted, geographic distribution, pathologic findings and data quality. Spatial and temporal patterns of submission were heterogenous for both species reflecting both natural events and artificial sampling bias. Sampling rates were low with a high degree of variability by season, year, location and nature of submission. Poor data quality reduced the amount of available data for analysis but quality dramatically improved over time as there was increased desire to use this system for surveillance. Measures to improve both quality and quantity of information would improve this passive surveillance system and its ability to detect disease.

* 1966-1995 – Western College of Veterinary Medicine- Department of Pathology
1996- 2006- Canadian Cooperative Wildlife Health Centre

3.2 INTRODUCTION

For over 40 years, wildlife pathologists in Saskatchewan at the Western College of Veterinary Medicine (WCVN) (52 Campus Drive Saskatoon, SK, Canada S7N 5B4) have been investigating morbidity and mortality events in wildlife through submissions of carcasses for diagnostic pathology. The primary objectives of these investigations have been to advise managers (wildlife and domestic), conservation officers and hunters on issues related to infectious disease, metabolic disease, food safety, and forensic science. Secondary to this, these submissions form what is described in the literature as a passive surveillance system which can be used to monitor populations over time and to detect and describe significant diseases. There is little dispute that these passive surveillance systems play an important role in disease discovery, however the efficacy and efficiency of these systems are highly variable and depend on a number of factors associated with both the disease itself and the strength of the surveillance system. Identifying limiting factors which reduce the sensitivity of a system is important for interpretation of surveillance outcomes and for strengthening surveillance systems.

In Saskatchewan, wild cervids, such as white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) make up a large proportion of the surveillance submissions. Both species are abundant and are a valued resource, contributing an estimated 3 million dollars to the local economy yearly in hunting revenues (Arsenault, 2005). Estimated populations for 2003 in the province were approximately 369 000 white-tailed deer and 43 000 mule deer (Arsenault, 2005). The ranges of the two species overlap; white-tailed deer have a large range, which covers most of the entire southern half of the province, while mule deer are more concentrated in the south-west (Figures 1 and 2).

Surveillance in Saskatchewan of these two species in particular, has led to several diseases being first described in Western Canada, such as necrobacillosis (Wobeser et al, 1975), poliоencephalomalacia (Wobeser and

Runge, 1979), and dermatomycosis (Wobeser et al., 1983). Necrobacillosis was discovered when an outbreak caused significant mortality in a localized region of the province (Wobeser et al., 1975). Dermatomycosis (ringworm) is highly visible and easy for hunters and conservation officers to spot. Polioencephalomalacia results in neurologic signs which typically concern observers. All three diseases had factors which increased the likelihood of being detected; they were highly visible because they affected a large number of animals, or were easy to recognize.

Unlike livestock populations, free-ranging populations are not under constant “vigilance”. The probability of observing a free-ranging animal that is ill or dead may be dependent on factors such as the size of animal, time of year and foliage cover. Further to that, vigilance is greatly affected by the distribution and density of the human population. In Saskatchewan, the population density is one of the lowest in Canada and is largely concentrated around urban centers. The majority of the rural municipalities average less than 1 person per square kilometer (http://esask.uregina.ca/entry/population_trends.html), resulting in a decreased likelihood of observing an animal in rural areas. Once an animal is “observed”, the training and awareness of the observer, along with their level of concern will impact their decision as to whether to contact wildlife authorities or not. Whether a wildlife authority responds or not may be a function of logistics, funding and level of awareness or education. The resulting spatial and temporal variation in surveillance “vigilance” may impair disease detection. For example, chronic wasting disease (CWD) was not detected through this surveillance system until 2005; more than 5 years after it was first discovered in free-ranging populations using hunter-harvest submissions (Trent Bollinger, personal communication). Further exploration of the submission patterns may provide insight into strengths and limitations of this system, including how to improve it for the purposes of disease detection.

The primary objective of this study was to characterize the amount and distribution of white-tailed and mule deer samples submitted from Saskatchewan to a provincial laboratory for diagnostic pathology from 1966-2006. A descriptive analysis was conducted to describe sample submissions by species, type of submission, sex, age, month of submission, geographic distribution, and pathologic diagnosis. The intent of this analysis is to describe patterns of submissions to this surveillance system, and how they may have changed with time. The use of this system to detect emerging diseases, such as was the case for CWD in the late 1990s and early 2000s will be examined. This information will also be used in the qualitative and quantitative evaluation of CWD surveillance systems.

3.3. MATERIALS AND METHODS

All records of cases originating in Saskatchewan from 1966-2006 submitted for pathology examinations to the Saskatchewan provincial diagnostic services¹ under the category white-tailed deer, mule deer, or deer (non-specified) were examined. A total of 2163 free-ranging deer were submitted by conservation officers, hunters, biologists and public patrons during that time period. Information obtained from the records was recorded in a database (Microsoft Office Excel 2003) and included information on species, nature and type of submission, history, date found, date submitted, age, weight, sex, body condition, geographic location, necropsy findings and pathologic diagnosis.

Routine post-mortem examination typically included species identification or verification, age and sex determination, and body condition scoring. Age was primarily determined using examination of tooth enumeration and wear, and varied with examiner experience and sample availability. The body condition score for carcasses was determined by the examining pathologist and was classified as (1) very good, (2) good, (3) fair, (4) poor, or (5) very poor.

¹ 1966-1995 – Western College of Veterinary Medicine- Department of Pathology
1996- 2006- Canadian Cooperative Wildlife Health Centre

All whole or partial carcasses received a thorough gross pathologic examination, followed by tissue/sample collection for histopathologic, bacteriologic, parasitic or viral evaluation in selected cases. In cases where only selected tissue samples were submitted, examination was limited to those tissues and a limited diagnosis was made.

Upon complete examination of the specimen, results were interpreted and a primary and secondary diagnosis (in selected cases) was given. In order to facilitate interpretation for the purposes of this study, the primary diagnoses were further grouped into the following categories: trauma, infectious/inflammatory, emaciation/starvation, unknown/no diagnosis, survey (various), poisoning toxicity, neoplasia, anomalies, and other.

For mapping purposes, samples were aggregated at point locations and mapped by species for the periods before CWD was discovered in free-ranging populations (1966-1999) and after (2000-2006). Kernel estimation was used to generate raster maps and account for point density at specific locations and demonstrate geographic concentrations or hot spots of sampling.

Geographic location where the animal was found was recorded and geographic coordinates (latitude/longitude) were assigned. All submissions with location information were mapped, accounting for multiple submissions originating from some locations. Samples were also categorized according to the quality of location information: 0) very poor; only location of natural resource office, 1) poor; general area (closest town) where the animal was found, 2) fair; specified area (distance and direction from closest town) where the animal was found, and 3) good; exact location provided (latitude/longitude coordinates or land location information).

Descriptive statistics were generated using SPSS for Windows, Rel. 14.0.0.2005.

Chicago: SPSS Inc.. Maps were generated using ArcGIS Desktop (Version 9.3. Redlands, CA: Environmental Systems Research Institute, 1999-2008).

3.4 RESULTS

A total of 2163 samples of free-ranging white-tailed deer, mule deer and “deer/non-specified” were submitted from 1966-2006. White-tailed deer comprised the majority of the submissions (1715 – 79.3%), followed by mule deer (317 – 14.7%) and “deer” non-specified (131 – 6.1%). The two free-ranging deer species present in the province of Saskatchewan are white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*), therefore those submitted under the category “deer/non-specified” were assumed to be of either species; however information on age, sex, and location obtained from samples submitted under “deer/non-specified” was of poor quality and therefore these records were excluded from the remainder of the analysis (n=131).

The number of samples submitted fluctuated yearly in both species. Sample submissions for white-tailed deer ranged from 1-227 per year with a median of 31 samples per year (Figure 3), while sample submissions for mule deer ranged from 0-37 samples per year with a median of 6 samples per year (Figure 4).

Information on the month when the animal was found was compared to the month submitted to determine if a significant amount of time often elapsed between the two events. Of those records with both dates provided, 1133/1275 (88.9%) were submitted = 31 days after they were found and 142 samples (11.1%) were submitted >31 days after being found. Therefore, because the date in which the animal was found (or shot) was missing for a number of submissions, date of submission was used as a proxy when estimating the season in which the animal died. The majority of samples were submitted from November through April (88.7% WTD; 82.3% mule) with few samples being submitted from May to October (late spring/summer/fall) (Figure 5).

Samples were examined to determine the age and sex distribution of submissions. A total of 1711 (84.2%) cases provided information about age (missing n=321); and 1845 samples contained information on sex. Results are compared (Table 1) with provincial age structure estimates based on annual Cooperative Deer Management Survey (CDMS) field observations (September – November) (Arsenault, 2005).

In total, there were 1515/1715 (88.3%) white-tailed deer samples and 275/317 (86.8%) mule deer samples with location information ranging from very poor to good quality. On average, the location information provided at time of submission was of low quality (Figure 6). The quality of location information substantially improved with time.

The maps of sampling density (Figure 7) reflect heterogenous sampling across time and space; partly due to the distribution of deer in the province, to specific events such as outbreaks, but also in part due to other human factors. Some conservation officers were very interested in participating in the mortality/parasite studies, thus sending in higher numbers of WTD samples from the Harris area (west central) in 1970-72 and the Hudson bay area (east central) in the years 1974-79. The WTD cluster in the SE corner in the Moose Mountain, Carlyle and Estevan area was due to multiple submissions over many years. There was no distinct reason discernible for these increased submissions; however it could reflect high deer density, natural events causing high mortality, or specific individuals with a keen interest in mortality events. After 2000, the spatial pattern of sampling changed. The discovery of CWD in a wild mule deer in Lloydminster area increased submissions from that area, and this is reflected in the map. For WTD, approximately 50% of deer submitted from the Prince Albert, Nipawin and Love areas were submitted in 2005 and 2006, after CWD was discovered in the wild in that region.

The five most common categories of pathologic diagnoses were: trauma 884(43.5%), infectious/inflammatory/metabolic processes 465 (22.9%), emaciation/starvation 254(12.5%), other 145 (7.1%) and unknown/no diagnosis 129(6.3%). Pathologic findings are summarized in Table 2.

Traumatic injuries were further categorized: motor vehicle collisions accounted for 46.3% of all traumatic injuries (n= 409), while gunshot wounds (n=147; 16.6%), and predators (n=136; 15.4%) were also significant causes of trauma. Generalized trauma of unknown origin was diagnosed in 192 (21.7%) cases.

The infectious/inflammatory conditions category was further stratified, with local inflammatory conditions such as abscesses being the predominant diagnosis (Table 3). Disease conditions such as dermatitis (dermatophycosis), necrobacillosis, rumenitis and polioencephalomalacia represent a significant proportion of submissions under this category and reflect study interests of researchers.

Clinical history was limited in most submissions; with the majority of submissions either “healthy” (shot by hunter) or found dead. There were a number of cases submitted with a history of neurologic signs (limited flight response, aggression, ambulatory difficulties, salivation). Historically, rabies has always been a primary concern in cases presenting with neurologic signs. More recently polioencephalomalacia (1990) and chronic wasting disease (2000) have been added to the list of differentials. These diseases are of increased concern to the public, and as a result, clinically neurologic animals are thought to have relatively high submission rates. The submission rates for white-tailed deer and mule deer exhibiting clinical disease with neurologic signs is given in Figure 8.

3.5 DISCUSSION

This retrospective review demonstrates the variability that can occur with passive surveillance systems and their resulting limitations. The greatest limiting factor of this passive surveillance system is sampling rate; rates in both species were

highly variable and overall, low. Submission rates were heavily impacted by both natural events such as outbreaks or harsh winters, but also by 'artificial' events such as special studies and surveys.

Rates of submission also were highly variable throughout each year. The marked seasonality of sample submissions (November through April) could reflect true increases in mortality but could also be due to observational bias. Previous studies suggest that mortality rates are much higher during these months, with increasing risk over the course of the winter due to harsh climatic conditions and resource scarcity (DelGuidice et al., 2002). Additionally, the primary hunting month is November which accounts for a significant amount of mortality, especially among adult bucks. Another explanation for seasonality of submissions is that there is an increased probability that the animal will be found and submitted with better carcass visibility and preservation in the colder months. Likely both observation bias and increased mortality contributed to the seasonality of submissions.

Bucks greater than 1 year of age were overrepresented in this sample, relative to the estimated age and sex distribution of the underlying population. This could reflect a true increase in mortality rates or alternatively could be due to observational bias, as hunters are more likely to shoot bucks, and therefore observe pathologies associated with them. Increased submission rates among bucks is somewhat expected due to increased hunting pressure, including forensic investigations and deaths secondary to non-fatal gunshot wounds.

The spatial distribution appeared to be less dependent on urban populations than originally hypothesized. After 2000, there does appear to be an increased number of WTD samples submitted from areas surrounding Regina and Saskatoon (the most populous cities in Saskatchewan), but overall the majority of hot spots were dependant on deer densities, disease discovery (CWD), surveys

and conservation officers. All these factors must be considered when interpreting surveillance findings.

The most commonly reported pathologic diagnosis was trauma. This included gunshot wounds, vehicular collisions, predation, and trauma of unknown origin. This is similar to radiotelemetry findings, where gunshot wounds, vehicular collisions and predation have been reported to be significant causes of mortality (Bleich and Taylor, 1998; Pusateri Burroughs et al., 2006). Following trauma, infectious/inflammatory disease was the second most common disease finding. This was largely due to hunter submissions of localized lesions for determination of wholesomeness of the carcass. The nature of submissions fluctuated over time, with more samples submitted with a history of neurologic disease following the identification of CWD in free ranging populations.

Data quality overall, was a limiting factor in this analysis and accounted for a significant reduction in the amount of data available. There are many factors contributing to this. In some situations, scavenging and degradation of carcasses prevented determination of age, sex, body condition or diagnosis. In other situations, the information was likely available, however was not recorded at the time of collection. The majority of samples (approximately 85%) appeared to have been submitted by conservation officers, although information on the type of submitter was not directly collected. In general, information obtained from hunters was of poor quality, likely due to the specific nature of their investigations. Often, hunters would only send the relevant portions of carcasses with pathology with specific questions in mind, and were less likely to provide information on species, age, sex, and body condition. Information received from surveys, such as winter mortality studies, was also of poorer quality. This could have been because multiple deer were often collected at once, and individual characteristics were difficult to keep organized. Alternatively, individual characteristics may not have been thought to be important, and therefore less

attention may have been taken towards noting details such as exact geographic location, because harsh winter conditions were consistent throughout a region. Overall, the poor quality of location information limited the extent of spatial analysis that could be conducted. Quality improved following the formation of the Canadian Cooperative Wildlife Health Center, and continued to improve with the development of chronic wasting disease surveillance programs. The quality of data improved considerably over time, as more effort was put towards using these records for surveillance purposes. Data quality has been identified as an ongoing challenge with current surveillance programs (Trent Bollinger, personal communication), and value of sufficient resources and attention put towards standardizing data collection can not be overstated.

Despite their limitations, passive surveillance systems can be a valuable source of information about the population under surveillance and have contributed to the discovery of important diseases. Where they seem to work best is in diseases with high prevalence in the population and/or that are highly visible and easily recognizable. Because this system relies on human observation, conditions which increase the likelihood of human interaction, both spatially and temporally, are more likely to be detected. This has been demonstrated here with diseases such as grain overload, rabies, poliоencephalomalacia, and chronic wasting disease. Diseases such as anthrax and epizootic hemorrhagic disease (EHD), which primarily occur in the warmer, drier months are less likely to be detected for a variety of reasons (Beringer et al., 2000). Increased temperatures would degrade the carcass faster, reducing both the quality of the specimen and the likelihood that an observer would approach it. There is also more foliage reducing visibility. Alternatively, diseases which are more likely to occur in the colder months are more likely to be detected, with better preservation of carcasses and reduced foliage. Vigilance also increases during hunting season, as more people are out observing deer (eg. Chronic wasting disease is more commonly reported in the fall and winter (Williams, 2005)).

Therefore, although chronic wasting disease spreads slowly and takes time to increase in prevalence, it is likely to be detected among passive surveillance because of the neurologic changes that occur in an infected deer. Clinical CWD animals are more likely to wander into yards (Trent Bollinger, personal communication) and less likely to avoid motor vehicle collisions and predator attacks (Krumm et al., 2005; Williams, 2005). The most likely reason that it was not detected early in Saskatchewan through this surveillance system is that there were simply not enough samples being submitted with consistency. In white-tailed deer, submission rates did not increase overall in 1996 (after CWD was discovered in farmed cervids) or in 2000 (after CWD was discovered in a wild cervid) but the nature of submissions did shift more towards animals acting abnormally. This shift is much more dramatic in mule deer, especially after 2000. Both submission numbers and submissions of animals acting abnormally increased following detection of CWD, reflecting increased 'vigilance' and concern for chronic wasting disease in this population.

CONCLUSION

With demands for wildlife surveillance information increasing and diversifying, laboratory submission-based passive surveillance systems, such as this one, could play an increasingly important role in disease discovery and monitoring as non-random sampling, or targeting of the "3D" (diseased, dead, dying) subset of the population can be a very efficient approach to detecting new diseases at low prevalence. In Saskatchewan, spatial, temporal and demographic submission patterns were variable and heavily dependent on specific, intermittent research projects. Although these projects were very valuable in providing resources that otherwise would not have been available for wildlife disease surveillance, they ultimately affected the surveillance outcomes. This system could be greatly enhanced by improving submission rates across all regions and acquiring better quality information. It follows that more consistent funding of surveillance of wildlife populations would dramatically improve these systems.

Figure 1 - White-tailed deer range map showing habitat and management units (Source: Saskatchewan Ministry of the Environment)

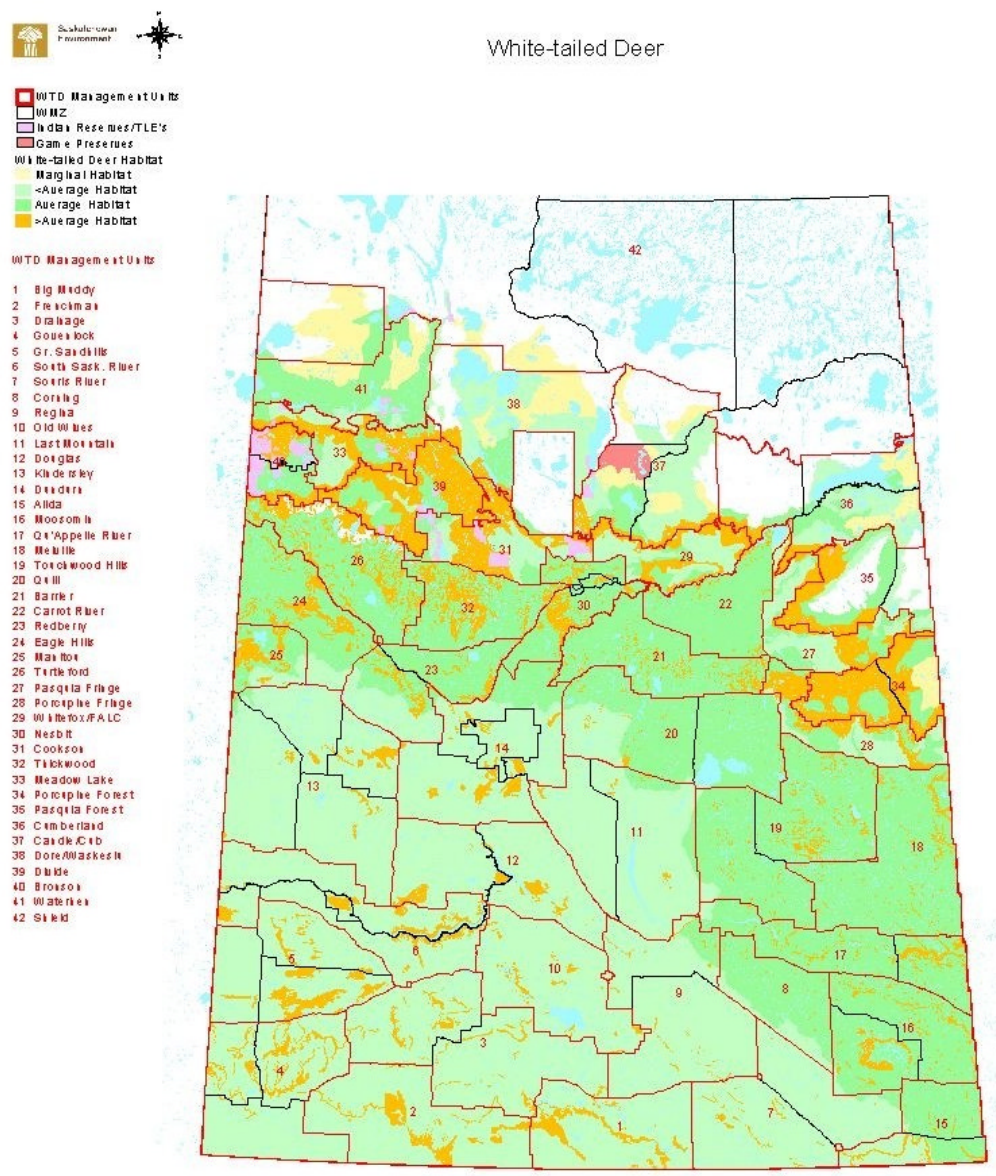


Figure 2 - Mule deer range map showing habitat and management units
(Source: Saskatchewan Ministry of the Environment)

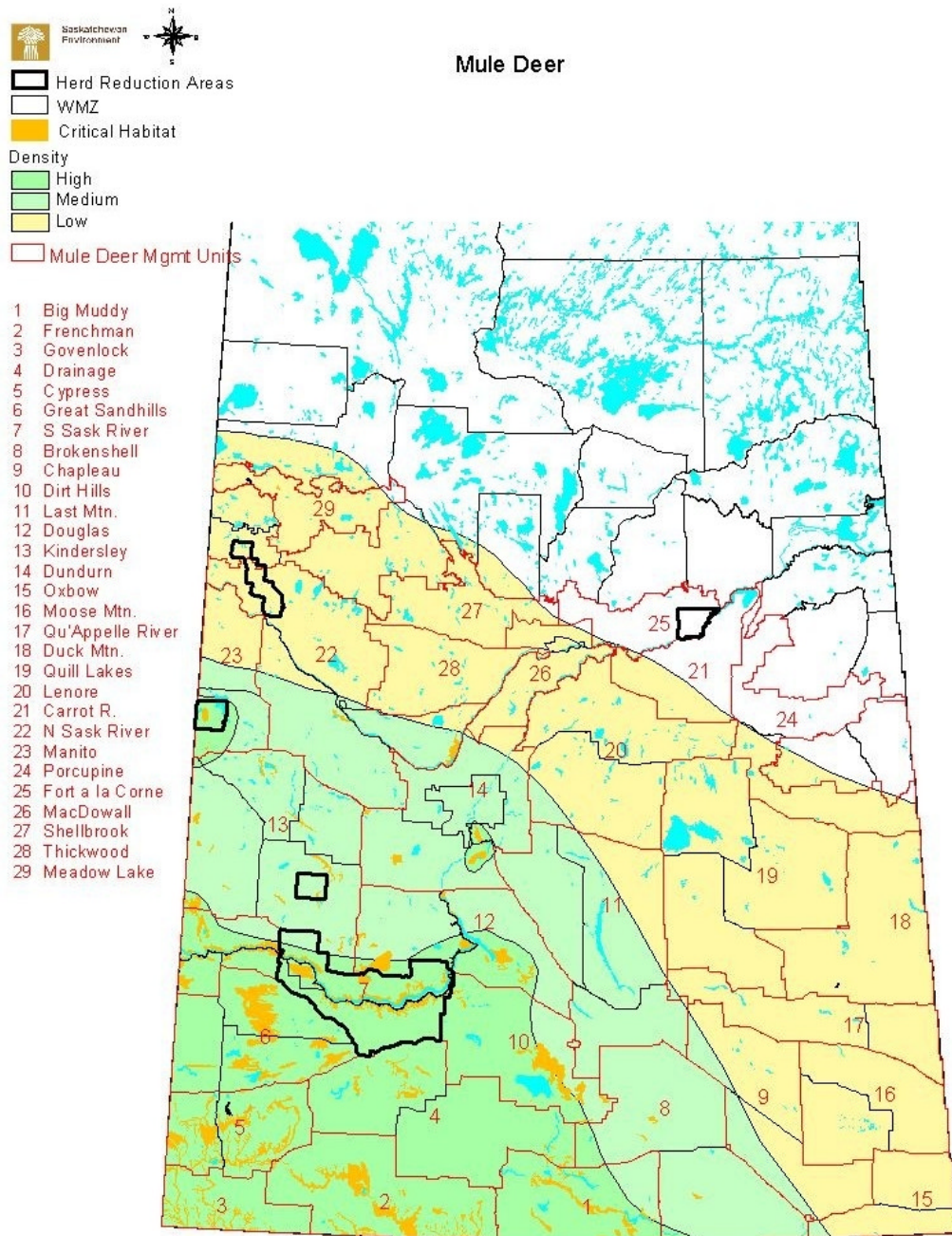
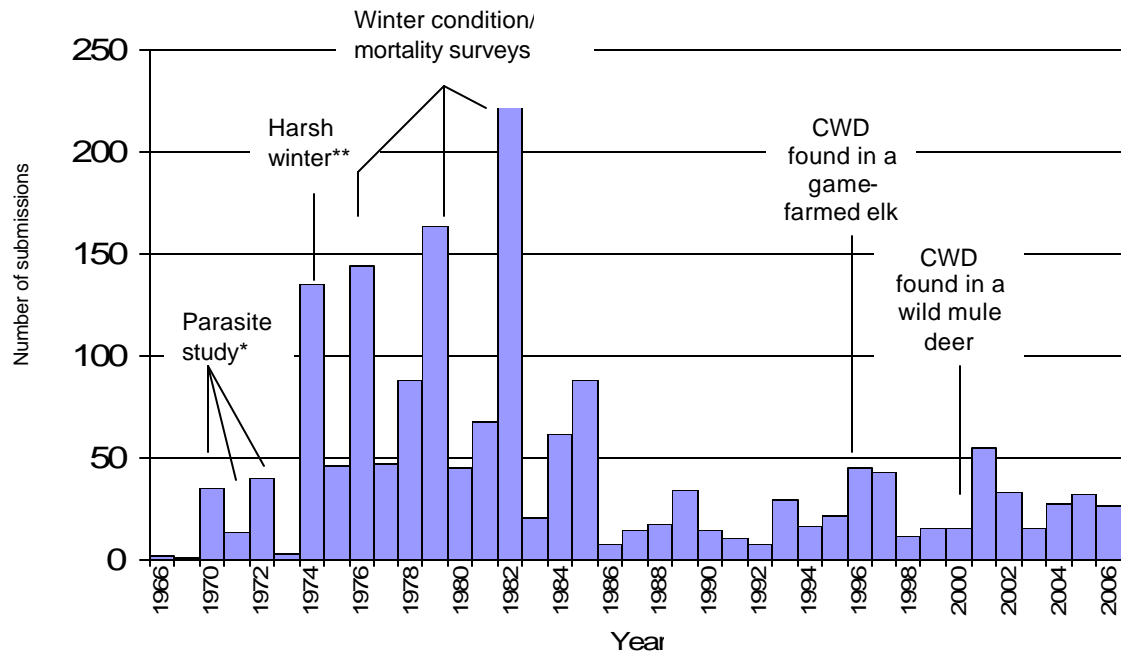


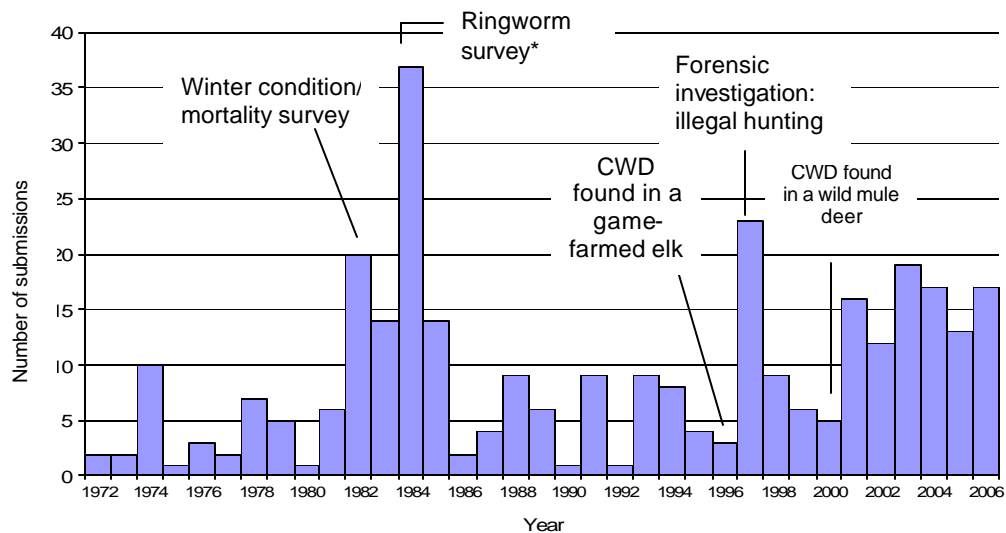
Figure 3 - Number of white-tailed deer samples submitted per year in Saskatchewan from 1966-2006



*study looking at parasite burdens of *Cephenemyia* spp., *Pneumostrongylus* spp.

**increased mortality due to starvation, necrobacillosis, grain overload and trauma

Figure 4 - Number of mule deer samples submitted per year in Saskatchewan from 1966 – 2006



* Targeted survey with hunter check stations looking for ringworm and fibropapillomas

Figure 5 - White-tailed deer and mule deer submissions from Saskatchewan by year and month

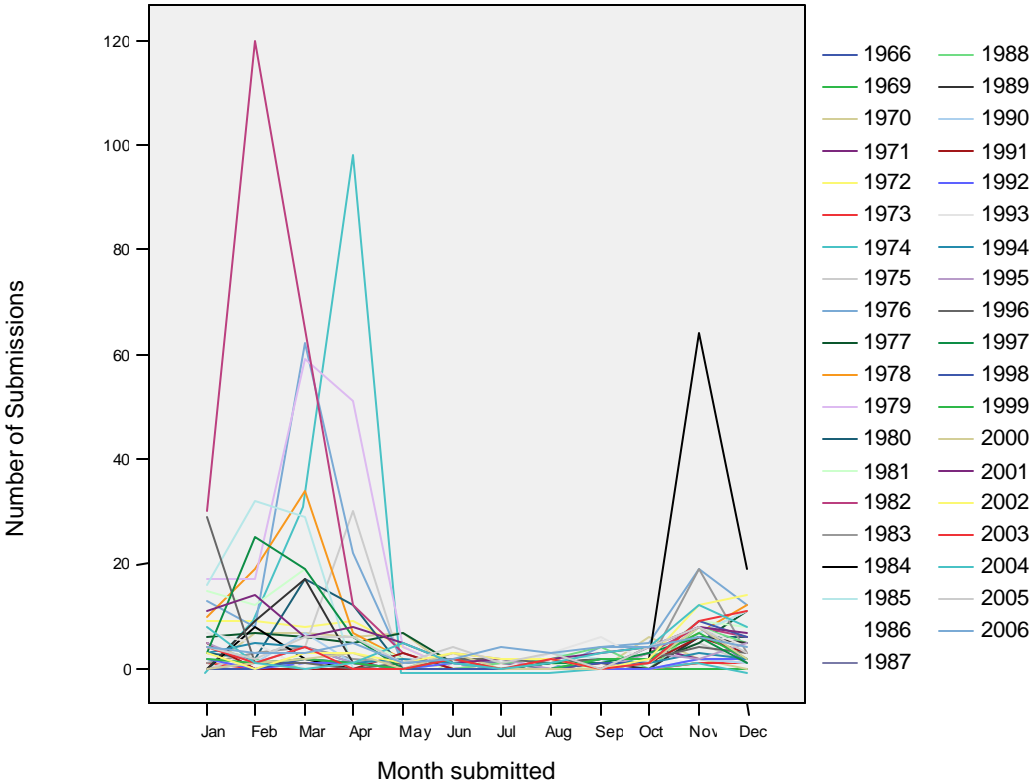


Table 1 - Comparing sex and age ratios of the pathology samples and the general population

RATIOS	White-tailed deer		Mule deer	
	Sample	Population	Sample	Population
Buck/doe	0.87	0.43 (Range: 0.32-0.47)	1.4	0.47 (Range: 0.38-0.55)
Fawn/doe	1.05	0.94 (Range:0.8-1)	0.81	0.79 (Range: 0.69-0.92)

**Figure 6 - Quality of location information provided at time of submission:
Proportion of very poor, poor, fair and good quality information by decade**

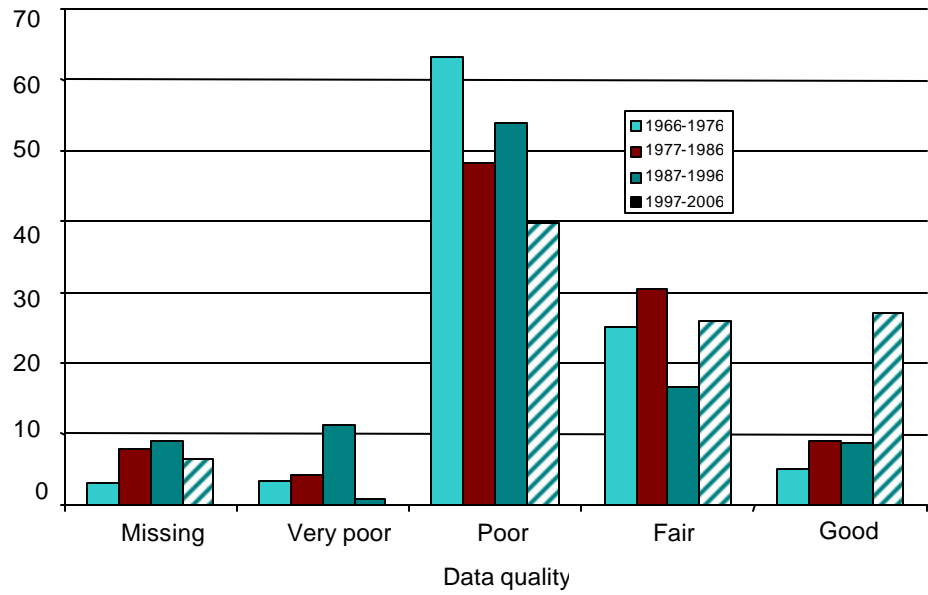


Figure 7 - Spatial distribution of white-tailed deer and mule deer samples submitted before (1966-2000) and after (2001-2006) CWD was discovered in wild cervid populations

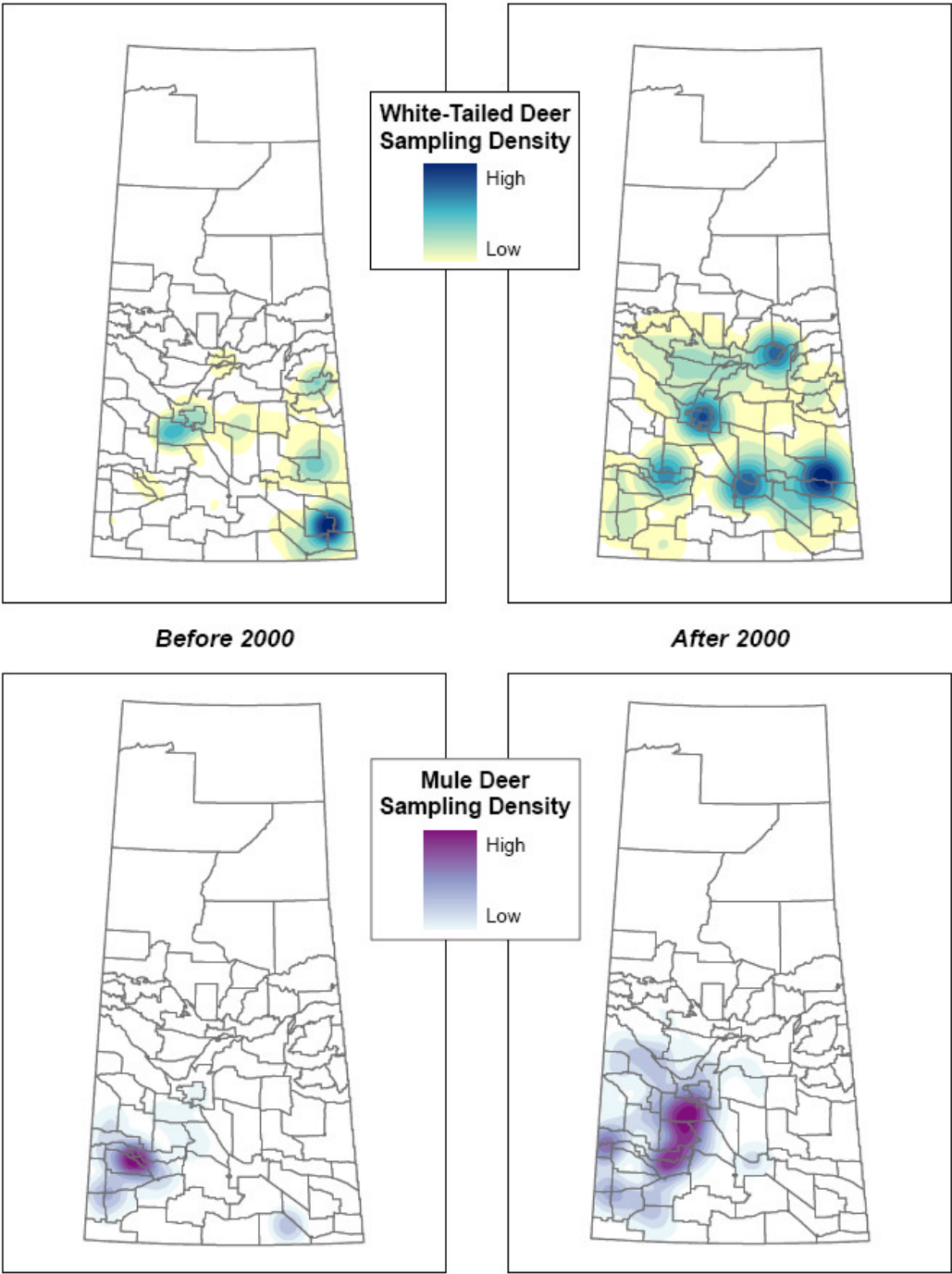


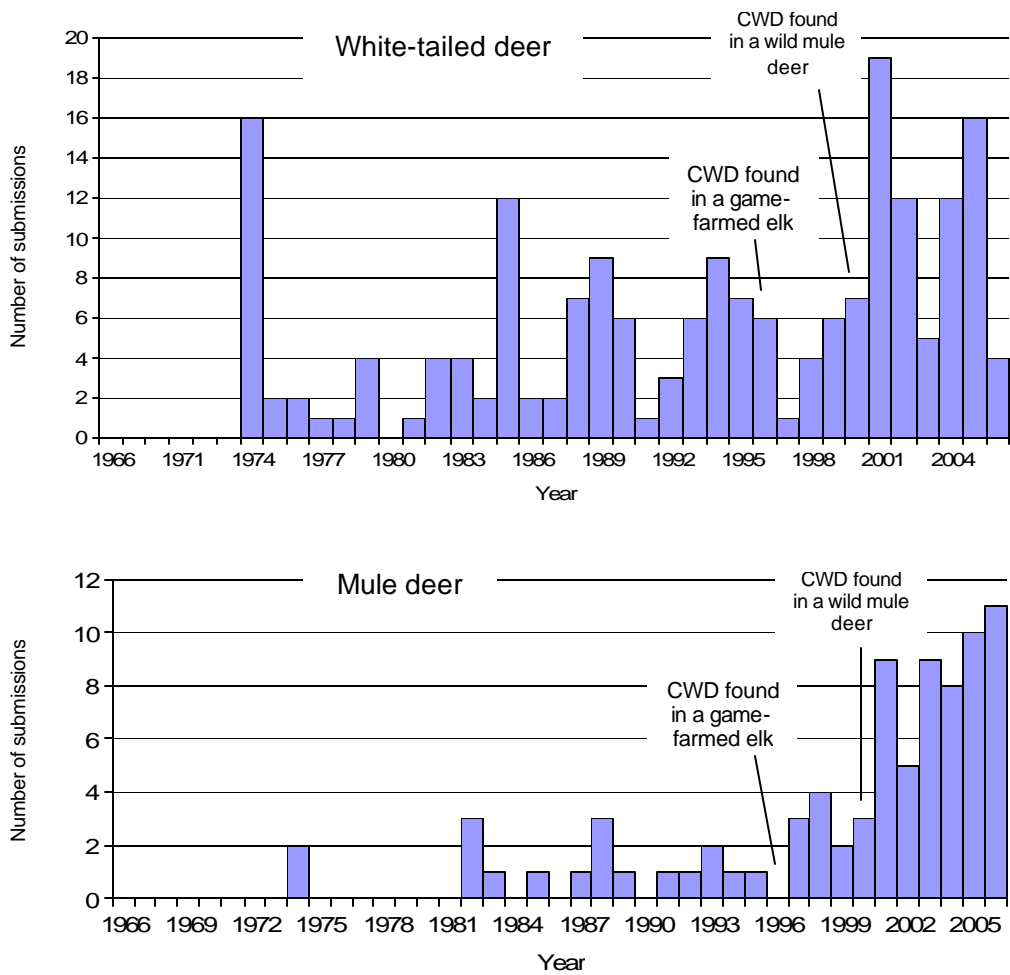
Table 2 - Pathologic diagnosis among white-tailed and mule deer submitted from 1966-1996

Pathologic Diagnosis	Frequency	Proportion
Trauma	884	43.5
Infectious/inflammatory	465	22.9
Emaciation/starvation	254	12.5
Other	145	7.1
Unknown/no diagnosis	129	6.3
Survey (various)	118	5.8
Poisoning/toxicity	23	1.1
Neoplasia	8	0.4
Anomalies	6	0.3
TOTAL	2032	100

Table 3 - Breakdown of infectious, inflammatory, and metabolic conditions by category of disease or specific condition

Diagnosis	Frequency	Proportion
Focal inflammation (various locations, eg. abscesses)	101	21.9
Dermatitis (including dermatomycosis)	59	12.8
Other systemic disease (various)	53	10.8
Necrobacillosis	48	10.4
Rumenitis/grain overload	44	9.5
Pneumonia	41	8.9
Encephalopathy (brain abscess, meningitis, and others)	35	7.6
Polioencephalomalacia	29	6.2
Fibropapilloma	28	6.1
Parasitism	22	4.7
Chronic wasting disease	4	0.9
Epizootic hemorrhagic disease	1	0.2
TOTAL	465	100

Figure 8 - White tailed deer and mule deer submissions with a history of abnormal behavior from 1966-2006



4. SURVEILLANCE SYSTEMS FOR CHRONIC WASTING DISEASE IN FREE-RANGING MULE AND WHITE-TAILED DEER IN SASKATCHEWAN: A QUALITATIVE EVALUATION

4.1 Abstract

A qualitative evaluation of chronic wasting disease (CWD) surveillance in free-ranging Saskatchewan mule and white-tailed deer was conducted in order to describe the surveillance system components, appraise the system's ability to detect CWD in new areas and monitor trends over time, and make recommendations to improve the system. The evaluation was conducted using information gathered from a descriptive analysis of surveillance results (1997-2006) and key informant interviews. The two main components of the surveillance system include hunter-harvested sampling of 'healthy' animals and pathology submissions of clinically ill animals (dead, diseased, dying- 3D). Hunter-harvested samples comprise the majority of the submissions and have detected almost all the CWD-infected animals to-date. Additionally, this surveillance component can be used to monitor trends in infected areas over time. Although the 3D surveillance component has not detected many cases, it is currently significantly limited by very low submission rates. Surveillance on clinically ill cervids, including CWD suspects, is a more efficient way to detect CWD in regions where the prevalence is low, and increasing submission numbers will greatly improve the sensitivity of this surveillance system.

4.2 INTRODUCTION

The demand for wildlife disease information has both intensified and diversified in recent years, resulting in the need to prioritize surveillance activities in the face of limited resources. The concept of ‘risk-based surveillance’ whereby diseases or issues that present higher risks merit higher priority for surveillance resources has been widely accepted and with this comes the need for surveillance systems that are both efficacious and efficient at meeting stated objectives. Regular, systematic evaluations of surveillance system attributes can help to ensure surveillance systems are meeting objectives and identify any areas where improvements can be made. In Canada, chronic wasting disease (CWD) was first discovered in elk in 1996 on a Saskatchewan game-farm. Surveillance began in free-ranging deer in earnest in 1997 in the areas surrounding the positive game farm to determine if the disease also existed in wild populations. Surveillance activities later expanded, and in 2000, a CWD-positive free-ranging mule deer (*Odocoileus hemionus*) was found. A formal evaluation of CWD surveillance in Saskatchewan has never been conducted. This evaluation will briefly detail the background on CWD, describe the current CWD surveillance objectives and components for free-ranging mule (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*), evaluate the attributes of the surveillance system components, and provide recommendations based on the findings.

4.3 BACKGROUND

Chronic wasting disease (CWD) is a fatal, infectious, neurodegenerative prion disease (transmissible spongiform encephalopathy; TSE) that affects both free-ranging and captive cervid species in North America. It was first identified in Colorado, USA in captive cervids as early as 1967 (Williams and Young, 1980). Since that time, the continent has watched the apparent geographic spread of this disease; efforts to control CWD have thus far been largely unsuccessful (Figure 1).

Current geographic distribution of CWD in Saskatchewan free-ranging deer populations

At the time when CWD was initially discovered in free-ranging populations in Saskatchewan (2000), it was believed to be a localized outbreak resulting from a spill-over from the infected game-farm (Trent Bollinger, personal communication). From 2000-2006, CWD was identified in 114 wild mule deer (*O. hemionus*) and 36 white-tailed deer (*O. virginianus*), primarily in 3 regions in the province near the Lloydminster, Saskatchewan Landing and Nipawin areas (Figure 2).

Importance for free-ranging and game-farmed deer populations

Chronic wasting disease in free-ranging populations has affected both the free-ranging populations themselves and the trade and production of captive cervids. CWD doesn't appear to be self-limiting and the long-term affects are predicted to result in a significant decline in deer population numbers (Miller et al., 2000). Additionally, a decline in deer populations would result in decreased hunting revenues which ultimately would reduce available resources to support wildlife management programs under current funding conditions. CWD has also had an impact on trade of Canadian captive cervids and their products, resulting in a dramatic decline in the value of the industry (Kahn et al., 2004).

Implications for human health

The transmissibility of CWD to humans via dressing of carcasses and consumption of infected meat and organs is unknown. There is currently no strong evidence to suggest that humans are susceptible to CWD (Belay et al., 2001; Belay et al., 2003; Belay et al., 2004; MaWhinney et al., 2006), however as with BSE, it is possible that humans are highly resistant to CWD infection, but still susceptible. And so, the precautionary principle is used regarding CWD's potential risk to humans. It is advised that hunters not harvest animals that appear ill, and that they wear protective gear when dressing carcasses, avoiding contact with high risk tissues (brain, lymph tissue and spinal cord). It is also

recommended that any tissue from a CWD-infected deer or elk is not used in animal or human food (WHO, 2000).

Controlling the spread of CWD

Disease control activities in multiple jurisdictions including Saskatchewan, and Alberta, currently rely primarily on density reduction. It is felt that delays in CWD detection in new areas would facilitate further disease transmission by allowing for the exposure of adjacent susceptible populations.

The Canadian Cooperative Wildlife Health Center and the Canadian National Chronic Wasting Disease Strategy

The Canadian Cooperative Wildlife Health Center (CCWHC) was created in 1992 in order to ensure a collaborative and coordinated approach to wildlife disease surveillance, research and response in Canada. In response to increased concern about CWD in Canada, the Canadian National Chronic Wasting Disease Strategy (CNCWDS, 2005) was developed to identify common goals in dealing with CWD, and to provide a coordinated national policy and disease management framework. 'The ultimate objective of the strategy is to eradicate CWD from Canada, or failing this, to achieve the tightest possible control of the disease so that it does not spread to new geographic areas or to new species, in order to minimize environmental, economic and health impacts' (CNCWDS, 2005). The six primary goals of managing CWD in Canada, as outlined by the CNCWDS are: 1) prevention, 2) early detection, 3) planned response, 4) effective disease management 5) education and training and 6) communication.

Challenges for CWD surveillance in Saskatchewan

At the time the strategy was developed, CWD was considered to be present at a low prevalence in a relatively small, defined geographic region; and as such, surveillance efforts were largely concentrated in that region. As the geographic range continues to increase, there is increased demand on the surveillance system, effectively thinning out resources across a larger geographic area.

4.4 SURVEILLANCE EVALUATION OBJECTIVE

To evaluate CWD surveillance in white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) in Saskatchewan and assess if current CWD surveillance objectives are being met.

4.5 CWD SURVEILLANCE AUTHORITIES AND STAKEHOLDERS

The data collected on the status of deer populations in Saskatchewan is primarily used by wildlife management organizations (Saskatchewan Ministry of Environment (SMOE), Canadian Wildlife Service, Parks Canada), hunters, domestic livestock organizations (Saskatchewan Agriculture and Food, Canadian Food Inspection Agency), wildlife research groups (Canadian Cooperative Wildlife Health Centre (CCWHC), University of Saskatchewan, University of Alberta), the provincial laboratory (Prairie Diagnostic Services (PDS)) and public health organizations (Public Health Agency of Canada, Health Canada). Hunter-harvested sampling is coordinated and funded primarily through Saskatchewan Ministry of Environment. All testing is conducted by pathologists in the Canadian Cooperative Wildlife Health Centre, in cooperation with Prairie Diagnostic Services. Results and demographic information are organized and stored in the CCWHC databases.

4.6 METHODS

The surveillance system evaluation was conducted, using a format and criteria previously outlined by Klaucke et al. (1988) and German et al. (2001). The evaluation consists of three parts: 1) describing or profiling the surveillance system in terms of objectives, purpose, system components, case definitions, outputs and resources, and 2) conducting a performance evaluation using the desired system attributes (simplicity, flexibility, data quality, acceptability, sensitivity, predictive value positive, representativeness, timeliness, and stability) and 3) synthesizing information in order to make conclusions and recommendations.

In order to describe and evaluate the surveillance system components, interviews were conducted with key stakeholders. The stakeholders included: the Canadian Cooperative Wildlife Health Center's CWD surveillance program manager, the program data manager, the Director of Policy, Finance and Administration, the IT Manager and Saskatchewan Ministry of Environment. CCWHC diagnostic laboratory reports and CWD testing results were also reviewed.

4.7 OBJECTIVES FOR CWD SURVEILLANCE IN FREE-RANGING MULE AND WHITE-TAILED DEER *(as outlined in the Canadian National CWD Strategy):*

- 1) Early detection of CWD at a low prevalence in cervid populations in order to rapidly intervene and to maximize effectiveness of control measures, including minimizing costs and economic losses.
- 2) To gather information on CWD in infected populations to assist in management decisions including monitoring the spatial and temporal trends in prevalence, disease transmission dynamics and the impact of disease control efforts.
- 3) To identify critical areas for further research in CWD to assist with early detection and effective management of this disease.

4.8 THE EVENT UNDER SURVEILLANCE

For the purposes of this evaluation, the population under surveillance is all white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) in Saskatchewan. A positive surveillance outcome is the detection of a CWD-infected mule or white-tailed deer in Saskatchewan. Immunohistochemistry is the current gold standard for CWD testing, and a case is considered to be CWD-positive if any neural or lymphoidal tissue stains positive. The target tissues most commonly tested include the tonsil, retropharyngeal lymph node and obex.

4.9 COMPONENTS OF THE CWD SURVEILLANCE SYSTEM

Prior to 1997, there was not a surveillance system in place specifically for CWD. There was, however, a passive multi-disease surveillance system that relied on

submissions of clinically ill (dead, diseased, dying) animals; this passive surveillance system is still in place. In 1997, in response to the discovery of the CWD-positive game farmed elk, a small hunter-harvest survey was conducted in areas surrounding a CWD positive game farm, with negative results. Regional hunter surveys continued through 1998 and 1999, and in 2000, a free-ranging mule deer was diagnosed with CWD. After this discovery in 2000, the hunter-harvested surveillance component was substantially expanded. Today, this is the primary means of conducting CWD surveillance. Currently, the surveillance system in place for chronic wasting disease (CWD) in wild cervids consists of these two components: 1) clinical surveillance which involves testing dead, diseased or dying animals (3D) that are found by members of the public, hunters or conservation officers, and 2) hunter-harvest surveillance, whereby hunters are asked to submit heads from harvested animals for CWD testing (Figure 3).

Component 1: Clinical surveillance via diagnostic pathology submissions (Diseased, dying, dead; '3D')

This is a convenience sample, with no specific sampling plan. Mule deer and white-tailed deer that are found dead, diseased or dying can be submitted any time of year to the Canadian Cooperative Wildlife Health Center for diagnostic pathology. These cases are acquired primarily through conservation officers, however hunters, farmers, biologists and members of the public submit a small proportion directly. Deer are more likely to be submitted in areas of increased human density, where they are more likely to be found. The decision to submit a sample for diagnosis is highly variable and depends on the judgment made by the conservation officers. Those deer with an apparent cause of death (eg. laying by the side of the road and therefore likely to have been hit by car) are less likely to be submitted than those with no obvious cause of disease or death. There is also a temporal variation in submissions; fewer carcasses are submitted in the summer and early fall compared to winter and spring for a variety of reasons (Chapter 3). The predominant reasons that conservation officers submit samples are: to determine cause of death, to diagnose disease agent, to determine wholesomeness of the carcass, and for forensic investigations. Once the

examination is complete, all results are entered and stored in the National Wildlife Disease (NWD) database housed within the CCWHC. The minimum data set within the NWD database consists of date submitted, name of submitter, location found, species, body condition score, age, sex, and gross pathological examination results. Other case information may be available such as history, date died, or results from advanced diagnostic examinations (histopathology, bacteriology, virology, parasitology, immunology, CWD testing) may be available on a case by case basis. CWD testing is now (as of 2005) conducted on all clinical submissions aged 1 year or greater. The results from CWD testing are stored in a separate database (CWD database) with linkages to the NWD database.

From 2000-2006, 4 positive deer were identified through this surveillance component.

Component 2: Hunter-harvested CWD surveillance

Hunters are required to submit the harvested heads through their local MOE regional office or designated drop-off location during and following the hunting season. In some areas, hunters may receive additional hunt tags after their initial deer heads are submitted. Received samples are tagged and demographic information (sex, age, location, date shot) is manually entered into a computer database. Location information is collected either descriptively (hunter describes where the deer was shot), or by a computer map, where the hunter simply points to the location on a computer screen and mapping coordinates are generated. In most cases, there is no information collected on the body condition of the animal, however if the animal is thin, the hunter will submit the entire carcass for post-mortem examination (under 3D surveillance) and a new tag will be issued. The information collected from the hunters is compiled and reviewed daily and then transferred electronically via an access database to the CCWHC, where it is integrated into the main CWD database. All samples are kept frozen until they are transferred to the CCWHC for testing. Samples collected are heads including teeth (for age determination), brain, tonsils, and retropharyngeal lymph nodes

(RLN) which then are removed by the CCWHC for testing. Once the tissue samples are extracted, half the tissue is frozen and half is formalin-fixed. They are packaged, numbered to maintain identification and stored until testing. Initial IHC testing is done on tonsillar tissue by Prairie Diagnostic Services; six to a cassette. A positive tonsil sample will trigger further testing of the obex and RLN. This allows for confirmation and staging of the disease. Formalin-fixed tissue which tests negative is discarded. All frozen samples (both positive and negative) have been retained in a tissue bank for future research purposes. Any samples which are not deemed to be testable (too autolyzed, or destroyed by gunshot) are discarded.

From 1997 to 2004, testing was restricted to certain regions. Outside of those regions, hunters were charged \$90 per head which dramatically affected sample submission rates in some areas. Since 2005, all harvested mule deer and white-tailed deer over 1 year old are eligible for CWD testing at no charge to the hunter, regardless of sex or location of harvest. Samples are collected primarily in the winter months of November and December, coinciding with the legal hunting season. Sample size depends on geographic region (license allocations determined by the Ministry of Environment and based on CWD prevalence, deer density and other factors). Areas where CWD is known to exist will tend to have larger sample sizes because of management decisions to reduce herd densities.

From 2000-2006, 145 positive deer were identified through this surveillance component.

4.10 CANADIAN COOPERATIVE WILDLIFE HEALTH CENTER

Data from hunter-harvest CWD surveillance is housed in the CWD database, while data from the clinical diagnostic specimens are housed in the National Wildlife Disease (NWD) database. A planning session was held in April 2007 to identify needs of data users, and to assist in directing improved functionality. As

a result, these databases were updated as of October 2007 and January 2008, respectively. One new application includes more drop-down menus which help to reduce data entry errors. There is currently no data dictionary available to users to standardize data entry. To date, no systematic review of data quality and data errors has been performed. These databases are backed up nightly. Access to these databases is limited and can be customized to the users needs (read only vs. full access). In general, raw data access is limited to CCWHC associates and to a limited number of researchers. Other requests for data are dealt with on a case-by-case basis. Data users must identify the intended use and sign an agreement for accountability, privacy and to protect intellectual property. The turn around time for a data request depends largely on the type of request made; these data are provided as an export file.

RECOMMENDATION: Regular reviews of data quality (ie. Describing the proportion of missing fields and data entry errors) would help to further characterize where drop-down menus, standardized codes and other data entry tools would improve data quality. Development of a data dictionary would help to catalog the organization and contents of a database, including describing how data elements are encoded. This helps to establish consistency.

4.11 CASE DEFINITION

A confirmed case is defined as:

A free-ranging mule deer (*O. hemionus*) or white-tailed deer (*O. virginianus*) with or without symptoms consistent with clinical CWD with one or more of the following tissues positive by immunohistochemistry:

- tonsil
- retropharyngeal lymph node
- obex
- any other lymphoidal or nervous tissue

Test results are reported by species and by geographic region. There is no stratification based on pre-test probability of positive results.

RECOMMENDATION: The case definition could be further expanded to incorporate the risk of being CWD positive and acknowledge the higher probability of disease in higher risk categories. Animals which exhibit symptoms consistent with CWD are more likely to test positive for CWD than animals without symptoms of CWD. Reporting results by risk profile will help to further

characterize any benefits of targeted testing. This could include the following case definitions:

1) CWD SUSPECT - any animal greater than 1 year from any region in Saskatchewan observed to have been in poor or very poor body condition with accompanying clinical CWD signs (salivation, depression, increased or decreased flight zone, low head carriage, teeth grinding, unable to rise).

2) ELEVATED CWD RISK - any animal greater than 1 year shot or found dead from any region in Saskatchewan and observed to be in poor or very poor body condition.

3) LOW CWD RISK- any animal greater than 1 year shot or found dead and observed to be in fair, good or very good body condition.

These case definitions are based on previous observations and published literature. Thin animals exhibiting clinical signs of CWD have a higher probability of being infected (Miller, 2000). Animals with clinical disease are also assumed to be at higher risk for death due to motor vehicle collisions, gunshot wounds, predators, aspiration pneumonia and sudden death (Krum et al., 2005; Conner et al., 2000; Williams, 2005).

4.12 OUTPUTS FROM THE SYTEM

Provincial reports of surveillance results by species and wildlife management zone (WMZ) are generated along with a map of the spatial distribution of CWD positive cases in the wild. These summary reports are displayed on the CCWHC website (http://wildlife1.usask.ca/en/cwd/chronic_wasting_disease.php). The website is designed to continually upload new results from the database as they become available; this allows for real-time access to the most current summary report throughout the testing season. After testing is completed for the season, the results are reviewed and some data cleaning may occur. In this case, the website will adjust its' results following the next upload. Other reports that are generated from this data are intermittent, but have included updates in the CCWHC newsletter, and surveillance and research updates for landowners and interested parties.

RECOMMENDATION: Providing a statement on the iterative nature of the summary report will notify report users that results are subject to change. This could also include labeling of the summary report as either a draft or final summary.

4.13 CASE FOLLOW UP

All positive CWD cases are reported to SMOE and to the Canadian Food Inspection Agency (CFIA). SMOE then notifies the submitter (hunter). Additionally, any hunter who submits a sample is able to receive his or her confidential results through a website application using their name and SMOE hunt tag #. The approximate turn around from time of submission to test results is 7-8 weeks. All positive samples must be sent for confirmatory testing to the CFIA.

4.14 OPERATING EXPENSES AND RESOURCES

When CWD was first found, Saskatchewan Agriculture and Food paid for the testing in free-ranging populations. Since 2004, SMOE has provided the funding for testing. The funding is provided yearly as a lump sum and covers costs related to sample preparation and testing. CCWHC offers human resources to support the program, including veterinary pathologists, data managers, administrators, IT support, technicians, and provides training opportunities. CCWHC also offers material support such as computers and lab equipment.

RECOMMENDATION: A cost-benefit analysis comparing the different surveillance components would assist program planning and ensure sound agency decisions based on benefits to the CWD program.

4.15 PERFORMANCE EVALUATION OF THE SURVEILLANCE SYSTEM

With the above stated objectives in mind, the most important attributes of this system are that it must be sensitive for early detection of disease and timely so that disease control action can be taken. Once CWD has been detected in a region, surveillance can be used to monitor trends over time and effectiveness of disease control efforts. It is then that the representativeness of the sample becomes important. Because both hunter-harvest and clinical surveillance components rely heavily on others to submit samples, it is also important that the surveillance system be simple and acceptable to users.

Sensitivity

Surveillance sensitivity can be viewed in multiple ways. Most often, sensitivity involves estimating the proportion of the total number of CWD cases in the population under surveillance that are being detected by the surveillance system. Because many diseases, including CWD, cluster within a population, surveillance sensitivity could also be defined as an estimate of the proportion of positive clusters (or herds) under surveillance that are being detected by the system. Detection of CWD is very challenging due to its long latent period, subtle and somewhat non-specific clinical signs (eg. emaciation), and initially low within-herd prevalence; it causes a protracted epidemic in a population, often taking years to increase significantly in prevalence (Miller et al., 2000). Because the prevalence remains low for a considerable amount of time, detecting CWD early in an infected herd requires a very large sample to be confident that the disease is not present (or present below an acceptable level). For example, if there are 1000 animals in the herd and the goal is to detect CWD at no higher than 0.5% prevalence (5 infected animals) using immunohistochemistry on tonsillar tissue (Se 99%; Spraker et al., 2002), at least 455 animals (46%) would need to be tested to be 95% confident that the herd did not have CWD at or above 0.5% prevalence (FreeCalc v2:

http://www.ausvet.com.au/content.php?page=res_software). This is neither feasible nor desirable in most situations for multitude of reasons. If the maximum acceptable prevalence is increased to 1%, a sample of 261 would be required and at 2% prevalence, a sample size of 140 would be needed. Even if a sufficient sample size is reached to detect a low prevalence of disease, this sampling would need to be repeated to account for the constant risk of introduction from surrounding infected areas or infected game farms.

Because CWD clusters geographically, the total population should be thought of as multiple population units. Within these units, a minimum sample size would need to be tested to be confident that the disease is present below the maximum acceptable level. In Saskatchewan, as is the case in many jurisdictions, the

province is divided into administrative units or wildlife management zones (WMZ). WMZs are the foundation for other geographic units, such as the MOE's management units and ecozones and have been also used to derive population estimates, making them somewhat useful for spatial analysis. One limitation of WMZs is that they do not necessarily correlate with biological population structures. Population estimates based on biologic population units would help to better interpret surveillance findings, however these estimates are currently not available.

As an example of sensitivity, mule deer surveillance samples harvested from WMZs 1-14 from 1996-2006 were examined in order to demonstrate the challenges in achieving good sensitivity for detecting CWD. Population numbers were estimated by mule deer management units (MDMU) using mean winter population estimates (1984-2003) generated by Saskatchewan Ministry of Environment (Arsenault, 2005). The sample size required to detect CWD at 1% and 2% prevalence for each MDMU was calculated using Free calc v2 (http://www.ausvet.com.au/content.php?page=res_software) and assuming test sensitivity of 99% (Spraker et al., 2002) (Table 1). Figure 4 depicts the actual number of hunter-harvest mule deer samples taken from these 7 MDMU from 1997 to 2006; approximate minimum sample size required to detect CWD at 1% (n=295) and 2% (n=150) prevalence is also given.

CWD was detected in two of these regions; 11-14 in 2002 and WMZ 8-10 in 2006. CWD has not been detected in any other zones, however only zones 2 and 4,5 have had intermittently high enough sample sizes to allow for conclusions about their disease status. Figure 4 also demonstrates the sampling bias towards zones where the disease has been found. This reflects the focus of this surveillance system on monitoring trends in infected areas, rather than disease detection.

RECOMMENDATION: Reporting surveillance findings, both positive and negative, accounting for underlying population in each region, would help to

better demonstrate where surveillance efforts are adequate to detect disease and where more samples are required.

One of the major challenges associated with the interpretation of these results is that the population estimates are based on administrative units, rather than biological units. This may lead to erroneous conclusions about the disease status of a population or adjacent populations.

RECOMMENDATION: A better understanding of the population distribution within the province on a finer spatial scale, including understanding the interactions and movement between and within species would help to define biological population units. These population units would increase the validity of sensitivity calculations and estimates of disease freedom of a population.

The value of target testing high risk animals has been recognized for some time. Animals with clinical disease (3D) have an increased probability of CWD infection and focused testing on this subset of the population will help to boost the sensitivity of the system. The challenge lies in the relative lack of methodology to analyze negative surveillance findings using non-random (targeted) sampling. Generally speaking however, if CWD were present in a population at 2% prevalence overall, the prevalence in the 3D population would be higher (see following section on sampling success) and the required number of animals to test to detect disease would be lower. Having recognized the value of target testing, there are many logistical barriers which often deter wildlife managers from pursuing this strategy. As a result, few samples are currently being submitted through this system and the current sensitivity of the clinical surveillance component is very low. Of the samples that are coming in, a number of them are coming from regions where CWD has already been detected. It follows that increasing submissions 3D cervids, particularly 'CWD suspects' and animals with '*Elevated CWD risk*' from areas where CWD *has not been detected* will increase the sensitivity of the surveillance system.

The sensitivity of the surveillance system components will be further explored using quantitative methods in a subsequent chapter.

Sampling success

The sampling success is defined as the proportion of samples that actually have the health-related event (CWD) and can be used to describe the relative value of further categorizing samples by risk profile. In the case of the hunter-harvest surveillance, which involves somewhat random testing of healthy animals, $145/28909 = 0.0050$ (0.5%) of samples were positive. With clinical surveillance $4/263 = 0.0152$ (1.5%) of cases were positive (Table 2).

If we instead stratify the deer tested by suspect case definition, elevated CWD risk and low CWD risk (hunter-harvest samples), the sampling success is increased dramatically in CWD suspects (Table 3).

Timeliness

There was very little difference reported in the timeliness of the two surveillance components (Table 4) and both components have acceptable timeliness. Overall, the time from submission to test results is usually around 3-4 weeks (up to 9 weeks) assuming a maximum of 1 week from sample collection in the field to submission to the lab. Often with harvested animals, hunters will wait for results prior to consumption. Positive carcasses are then incinerated and a new tag will be issued if applicable.

Because CWD is a slowly progressing disease, with a long incubation period seldom less than 18 months (Williams and Young, 1993; Williams, 2005), this surveillance system is considered by the author to be sufficiently timely for early detection of disease.

RECOMMENDATION: The performance of this surveillance system as it pertained to timeliness was only evaluated for early disease detection. There are however, other factors that could have been considered when measuring timeliness of this system, including timeliness of reporting results to hunters and other stakeholders. A survey could be conducted to assess whether this system is sufficiently timely to meet stakeholder needs.

Representativeness

Representativeness is how well a surveillance system describes the occurrence of an event over time and its' distribution in the population by place and person

(German et al., 2001). In CWD surveillance, it is important once CWD has been detected in an area for estimating true prevalence and following trends over time. For monitoring trends over time, hunter-harvest surveillance has been used in many jurisdictions as the best available approach. Although it is well understood that hunter-harvested samples have a propensity towards mature males and are not usually representative of the fawn and yearling population, in locations where CWD has been found, intensive herd reductions typically involve targeting of all age and sex categories. This results in a more representative sample. Hunter-harvest sampling is very efficient is likely the most efficacious. Sharp-shooting has also been used in some jurisdictions and is reportedly even better at detecting cases and targeting all age groups; however this strategy often has very little public support making it more difficult to implement (Margo Pybus, personal communication). Clinical surveillance results should not be relied upon to estimate prevalence as they are biased and not representative of the population (Miller et al., 2000).

This surveillance system would not be considered spatially representative, in that samples are clustered in certain geographic regions where CWD has been found, with relatively few samples coming from areas where the disease has not been detected, leaving the CWD status of these areas uncertain.

Simplicity and Acceptability

The simplicity and acceptability of the system was not formally evaluated, however some general comments can be made. The system is relatively simple; all samples are submitted through one wildlife authority agency who in turn submits the samples to one diagnostic lab for testing (Figure 3). Participation in the surveillance system is highly variable from region to region, and tends to improve following the discovery of a positive CWD deer. Testing is currently free for the hunter and individual confidential results can be retrieved easily online.

In 2004, only samples from known positive areas were tested at no charge. Samples originating from outside of positive areas were tested at a charge of

\$90. This caused a great deal of problems and submissions from “CWD-negative” areas dropped drastically. It was suspected that hunters were still harvesting from outside positive areas, but reporting that they originated within positive areas. This policy was quickly changed to the current situation, where all samples are tested at no charge.

Prior to 1996, all pathology records were stored in paper hard copy, and therefore required manual record retrieval. All pathology submissions (both domestic and wild) were received through the same intake system and were catalogued in sequential order as they were received, making record retrieval cumbersome and time consuming. All location information was in descriptive format and required geo-coding for mapping, which was also very time consuming. Since 1996, a searchable electronic database has been created to store all the record information making data storage and use much simpler. Improvements in standardization of data entry are being made, and since 2005, location information has been much easier to collect via computer mapping.

RECOMMENDATION: The surveillance system must be simple enough so that users of the system (SMOE, conservation officers, hunters) will submit as many samples as possible and provide the most complete information possible. There are a number of steps involved in the detection of a case. At each step, factors may impact whether a case is submitted or not. Methods for further improving the simplicity and acceptability of the surveillance system should be evaluated and barriers should be identified by consulting stakeholders to improve submission rates across all geographic regions.

Data Quality

Data quality speaks to the completeness and accuracy of the data recorded. Data quality of the clinical disease component has been discussed previously (Chapter 3) and overall the quality has greatly improved with time. Data quality of the hunter-harvest component is better than that of the clinical surveillance. There were 28909 hunter-harvested samples tested; only 123 (0.4%) were missing information on sex and 500 (1.7%) were missing age. Point location information (latitude/longitude or UTM easting/northing) was missing in 14326 cases (49.6%) but wildlife management zone information was only missing for

1719 cases (5.9%). Only 852 cases did not have any location information at all (0.3%). Therefore although the location information is reasonably complete, the spatial level to which the data can be analyzed is limited. Better point location information would allow for more fine spatial analysis.

The clinical surveillance data was of lesser quality; with 61 (14.9%) missing information on age and 160 (39.2%) missing information on sex. Point location information was missing for 308 (75.5%), while 23 (5.6%) submissions did not have location information at all.

RECOMMENDATION: Having poor quality data is very expensive as it requires extensive cleaning, limits the extent to which it can be analyzed, and increases the chance of errors in interpretation. The value of collecting complete and accurate data needs to be emphasized to all involved in data collection. Increased use of drop down menus, required fields and entry restrictions help to prevent typographical errors and reduce the need for data cleaning. Data dictionaries and definitions also help to describe data components and would assist in standardizing data entry. Because CWD spatially clusters on a relatively small geographic scale, good quality point location information should be the goal.

4.16 CONCLUSION

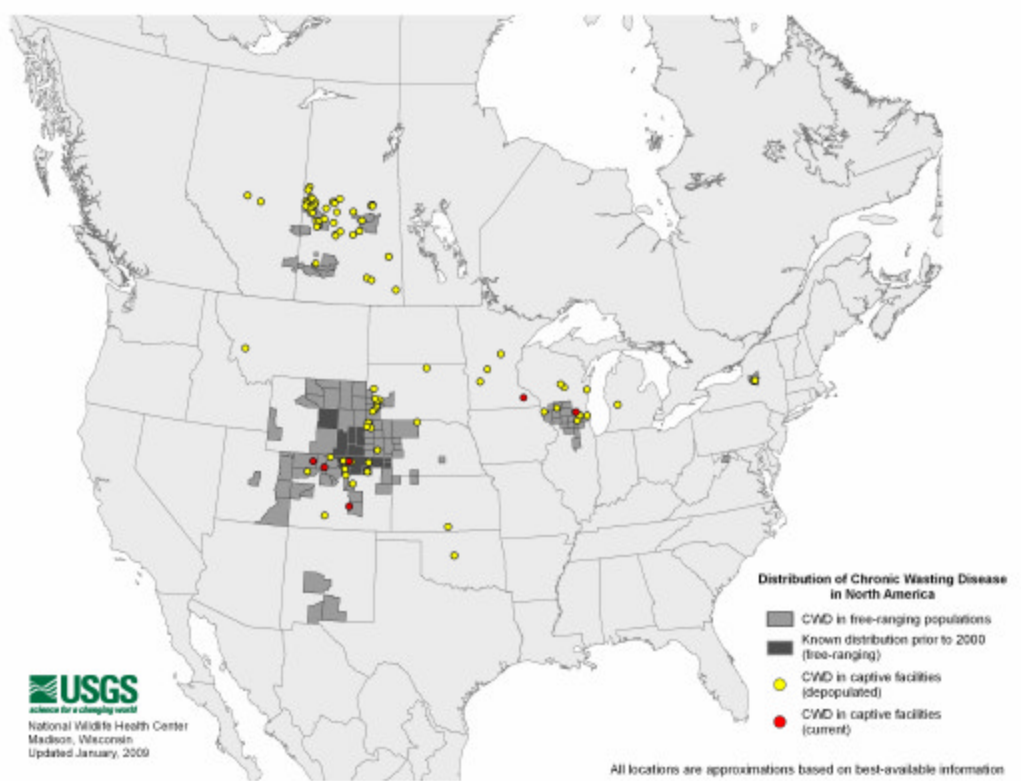
Chronic wasting disease surveillance in Saskatchewan must serve two main purposes: 1) early detection of CWD at a low prevalence for rapid intervention and 2) to monitor spatial and temporal trends over time. The current surveillance system places more emphasis on monitoring trends, perhaps at the expense of early detection. This is explained mostly by the coupling of disease control efforts and surveillance, in that harvests are heavily focused in CWD-positive areas. Additionally, because it is not recommended to consume CWD infected meat there is an obligation to provide testing to this group. As a result, a very large number of hunter-harvest samples have been tested and the majority of positive cases have been detected through this component.

In areas where the disease has not yet been detected and the prevalence is low, hunter-harvest surveillance is very inefficient at detecting disease. 'Healthy

animals' obtained through harvest have a very low probability of being infected and many more animals must be tested to detect disease. Alternatively, clinically ill animals, especially CWD suspects have a much higher probability of being infected and would therefore be a much more efficient way to detect CWD.

Although this is true in theory, there are many challenges that impair the sensitivity of this system that must be overcome for it to be effective. The current clinical surveillance component is not sufficient to fill in the gaps and the primary reason for poor performance has been a very low submission rate. Enhancing this system in areas where the disease has *not* been detected would improve sensitivity. This will likely require additional resources for education and awareness campaigns and for front-line staff, but given the importance of managing this disease, would be money well spent.

Figure 1- Distribution of CWD in free-ranging and captive cervids in North America, as of January, 2009

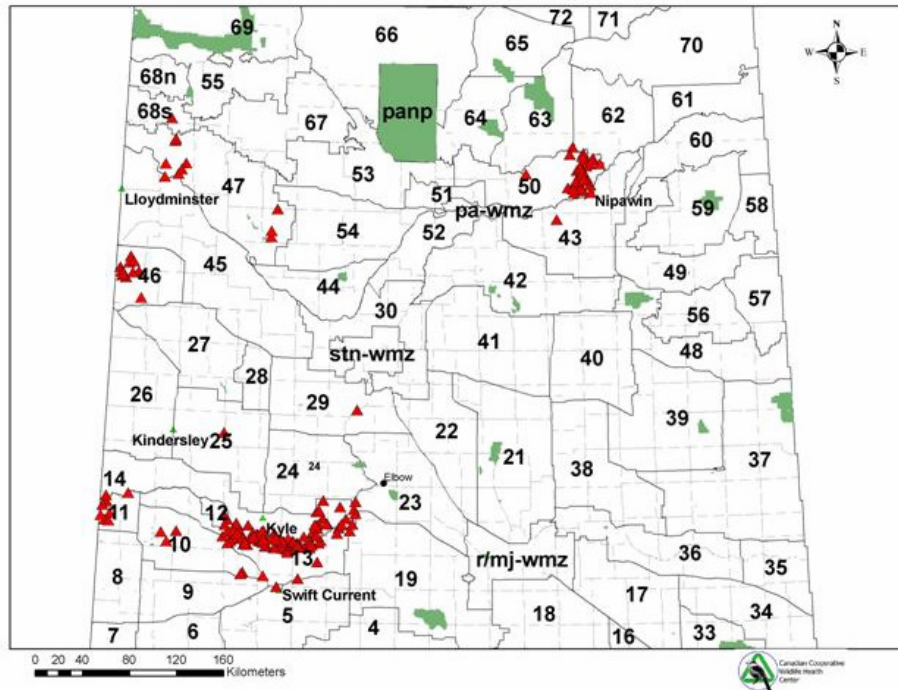


Source: http://www.nwhc.usgs.gov/images/cwd/cwd_map.jpg

Figure 2 - Locations of CWD positive wild deer (*Odocoileus hemionus*, *Odocoileus virginianus*, *Cervus elaphus*) in Saskatchewan 2000-2008

The Triangles Represent Locations of CWD Positive Wild Cervids in Saskatchewan, 2000-2008.

Numbered Areas are Wildlife Management Zones.



Source: http://wildlife1.usask.ca/en/cwd/CWD_All_Positives_2000-2008.jpg

Figure 3- Dual component surveillance system for CWD in free-ranging mule and white-tailed deer in Saskatchewan (component 1- deer with clinical disease or dead (3D); component 2- hunter-harvested deer)

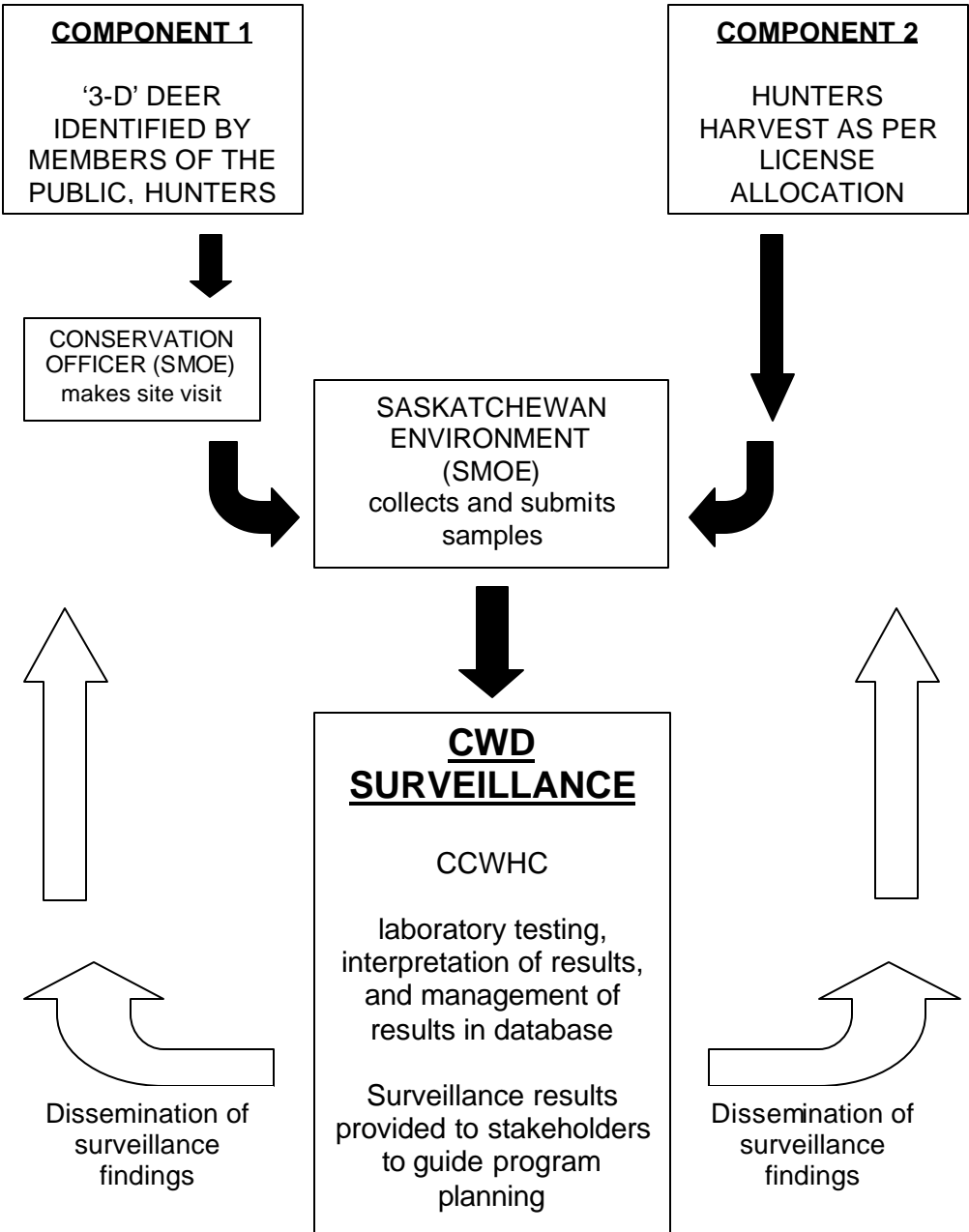


Table 1 – Mule deer population estimates in 7 southwestern mule deer management units (MDMU) (Arsenault, 2005) and required sample size to detect CWD at 1% and 2% prevalence

Prairie Grassland Ecozone				
Mule deer management unit (MDMU)	Wildlife management zone (WMZ)	Mean winter population (1984-2003)	Sample size required to detect 1% prevalence	Sample size required to detect 2% prevalence
Big Muddy	1	1528	278	141
Frenchman	2	3403	289	147
Govenlock	3	1532	279	142
Drainage	4,5	1509	275	137
Cypress	6,7	3315	290	144
G. Sandhills	8-10	8456	295	149
S. Sask River	11-14	9620	297	146

Figure 4 - Mule deer samples harvested from wildlife management zones (WMZs) 1-14 by hunters from 1996-2006 with the minimum sample sizes required to detect disease from these areas at 1% (n=295) and 2% (n=150).

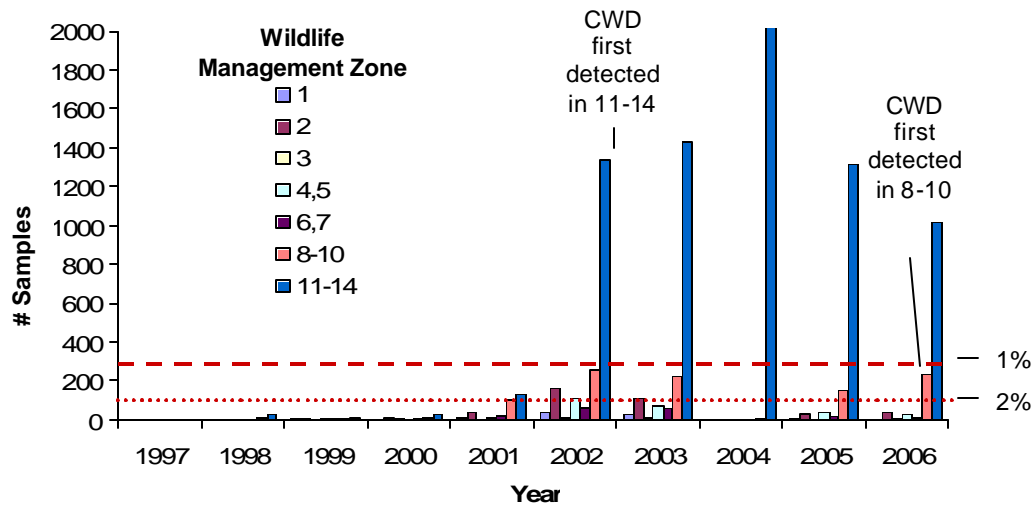


Table 2 – Sampling success of surveillance components 1 (clinical surveillance) and 2 (hunter surveillance)

	Component 1: Clinical surveillance since 1997	Component 2: Hunter surveillance since 1997	Total
# of positive cases detected	4	145	149
# of negative cases	259	28764	29023
Total # of cases tested	263	28909	29172
Predictive value positive	0.0152 (1.5%)	0.0050 (0.5%)	

Table 3 – Sampling success using risk profiles (CWD suspects; Elevated CWD risk; Low CWD risk)

	CWD suspects	Elevated CWD risk	Low CWD risk	Total
# of positive cases detected	4	0	145	149
# of negative cases	19	58	28946	29023
Total # of cases tested	23	58	29091	29172
Predictive value positive	0.174 (17%)	0 (0%)	0.0050 (0.5%)	

Table 4 - Time to detection of CWD in clinical or hunter-harvest surveillance components

STEP	EVENT	Components 1 or 2: Clinical or hunter surveillance
1	Animal is found sick or dead/ hunter harvests animal	0
2	Samples are collected	1day -1 week
3	Samples are submitted	1-2 weeks
4	Samples are tested	1-4 weeks
5	Animal status reported	1-2 weeks
	TOTAL TURNAROUND TIME	3- 9 weeks

5. A QUANTITATIVE EVALUATION OF CWD SURVEILLANCE IN FREE-RANGING DEER IN SASKATCHEWAN; 1997-2006

5.1 ABSTRACT

A quantitative evaluation of a surveillance system for Chronic Wasting Disease (CWD) in free-ranging mule (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) was conducted to evaluate surveillance system sensitivity and probability of disease freedom in the south-east part of Saskatchewan where CWD has not yet been discovered in wild populations. A scenario tree model was developed to incorporate target testing of animals with clinical signs of CWD along with hunter-harvested samples. A total of 120 clinically ill animals from the passive surveillance system and 2112 hunter harvested animals in 25 wildlife management zones (WMZs) from 1997-2006 were included in the analysis. Using this methodology, efficacy of the two surveillance system components was also evaluated. The overall mean sensitivity of both CWD surveillance components (*SSc_hunter* and *SSc_3D*) was poor from 1997 to 1999, increased dramatically from 2000 to 2003, and then declined to lower levels in 2004. Sensitivity results largely depended on the number of samples submitted and the design prevalence used in the modeling scenario. Low prevalence situations where it was desired to find one infected WMZ at 1% prevalence resulted in low sensitivity (22.7%; 95%CL:19%-26.4%; 2001) as compared to higher prevalence situations where it was desired to find two infected WMZs at 5% prevalence (79.4%; 95%CL: 75.8%-83.5%). Confidence of disease freedom was dependent on the ongoing risk of disease introduction. Based on surveillance findings, if the risk of introduction from 1997-2006 was low (0.01), the probability that CWD was present in at least 2 WMZs at 5% prevalence or greater was negligible. Finally, the relative value of testing 75 animals from the different surveillance components was compared to quantify the gain in sensitivity from target testing high risk CWD suspects. The sensitivity ratio was highest (12.537) under low prevalence scenarios (1 infected WMZ at 1%

prevalence), meaning in low disease prevalence situations there is much to be gained by using targeted surveillance.

5.2 INTRODUCTION

Chronic wasting disease was first detected in Saskatchewan in 1996 in a game-farmed elk (Kahn et. al, 2004). It was later found in wild mule deer (*Odocoileus hemionus*) (CCWHC, 2001), farmed and wild white-tailed deer (*Odocoileus virginianus*) (Kahn et. al., 2004; CCWHC, 2003) and was recently identified in wild elk (*Cervus elaphus*) (CCWHC, 2008). A map detailing the current known distribution of CWD in wild deer populations in Saskatchewan is given in Figure 1. Objectives for managing CWD in wild cervids, as outlined by Canada's National Chronic Wasting Disease Control Strategy (2005), include: 1) preventing further emergence 2) early detection and 3) effective management.

In Saskatchewan, surveillance for CWD in wild deer relies on hunter-harvest submissions, which primarily target healthy animals. Hunter-harvested animals are commonly used due to the relative ease in collecting large numbers of samples at a reduced cost. The sampling frame is not random; hunting is used as a means to manage populations and certain sex and age groups are more heavily harvested relative to others. The sampling rate and intensity is also spatially heterogeneous; sampling is largely tied to disease control and as a result, more samples are collected from regions where CWD has been previously discovered. In addition to hunter submissions, conservation offices or members of the public also submit diseased, dying or dead cervids (3D surveillance) for testing, including those that are exhibiting signs consistent with CWD (poor body condition with accompanying neurologic signs such as salivation, depression, increased or decreased flight zone, low head carriage, hyper-excitability, teeth grinding or unable to rise) (Williams, 2005). This population of clinically diseased animals is considered to be a high risk group, in that the probability of being CWD positive is much higher. Focusing on this subpopulation would be considered targeted or risk-based surveillance.

Surveillance for CWD is currently being conducted in both 'positive areas' where the disease has been previously identified and 'negative areas' where the

disease has not been identified. In areas where CWD has been found, the primary objective of surveillance is to estimate prevalence and monitor trends over time. Alternatively, in areas where CWD has not been found, early disease detection is the primary objective. When analyzing surveillance results from 'negative' regions, there are two possible scenarios to consider; negative surveillance could mean that the population is truly free from disease, or alternatively, that the disease is present in the population at some level but the surveillance system did not detect it. It is important to distinguish between these two scenarios.

The CWD strategy in Canada states that effective control hinges on early detection, and that the goal is to detect infected populations at no higher than 0.5-1% prevalence. At such a low prevalence, detection is extremely resource intensive; large numbers of randomly selected animals must be tested in order to detect infection, or alternatively to declare the population 'disease-free' with a certain degree of confidence (eg. 95% confident that CWD is below 1% prevalence). In the face of limited resources, risk-based testing strategies are a good alternative to large random samples. Risk-based testing (such as the '3D' surveillance) can be more efficient in detecting disease in that it targets animals (or populations) at increased risk of being infected (this subpopulation has a higher prevalence of disease). Similar to BSE, clinical "CWD-suspects" (greater than 1.5 years in poor body condition showing neurologic symptoms) have a higher prevalence of disease than the general population (Miller et. al., 2000). Additionally, these animals, through their altered mental status are more likely to get hit by a vehicle (Krumm et. al., 2005), killed by a predator (Williams, 2005), or to venture into farmyards (Trent Bollinger, personal communication). The challenge in using this non-random approach has been in the lack of methodology available to interpret negative findings (Samuel, 2003).

Quantitative methods have been developed to calculate disease freedom estimates using data derived from large random surveys; however few have

developed methodology that can incorporate multiple sources of disparate information, including information generated from non-random surveillance. Hueston and Yoe (1999) proposed using scenario trees to model surveillance systems to do power and sensitivity calculations. More recently, Cannon (2002) and Martin et al. (2007) presented methodology which allows for combining of multiple surveillance sources, including targeted surveillance programs to calculate sensitivity of surveillance systems and estimate the probability of disease freedom. Although this methodology has been used primarily in substantiating disease freedom claims for trade of domestic livestock, it was hypothesized that this technique could be extrapolated for use in other areas, such as CWD surveillance in free-ranging deer. Therefore, the objectives of this study were to apply methodology previously presented by Martin et al. (2007) to do the following: 1) To calculate the total mean sensitivity of the two CWD surveillance components in Saskatchewan ('hunter-harvest' and 'clinical' or 3D surveillance) in a 'CWD-negative' area from 1997-2006; 2) To estimate the probability of disease freedom in this area; and 3) To compare efficacy of the two CWD-surveillance system components.

5.3 METHODS

It is impossible to state that a population is absolutely disease-free without testing the entire population simultaneously with a perfect test. Rather, this methodology aims to determine probability of disease freedom given the sensitivity of the surveillance system at a pre-determined prevalence, or threshold prevalence (maximum acceptable prevalence) (Martin et al., 2007). Just like a diagnostic test, the sensitivity of a surveillance system is the probability that it will give a positive surveillance outcome, given that the population is truly infected ($Pr(T+|D+)$). Surveillance system sensitivity is dependant on the prevalence of the event of interest (CWD infection). Intuitively, if the prevalence of a disease is very high, fewer animals will need to be tested to detect the disease. Alternatively, with very low prevalence, many more animals would need to be tested to detect the disease. The threshold prevalence is often

determined by what is considered expected or acceptable. A commonly stated 'maximum acceptable prevalence' for CWD is 0.5-1%, meaning that the goal is to detect CWD in a population at 0.5-1% prevalence or lower (Bollinger et al., 2004).

This method uses stochastic scenario tree modeling to estimate sensitivity using multiple data sources, including non-random sampling. There are two primary assumptions: 1) all final results from the surveillance system are negative and 2) the specificity of the surveillance system is 100%, that is all results will be confirmed positive (distinguishing between false positives) (Martin et al., 2007). A scenario tree is developed to represent all the possible pathways from the starting point (population is infected) to the outcome (infection is detected or not) and to divide the surveillance population into groups which have the same probability of being diseased, given the population is infected. Groups are distinguished by factors which affect either their probability of being infected or of being detected. Each branch of the tree is assigned probabilities for each outcome, which helps to capture variable risk within a population. The surveillance sensitivity is then calculated by multiplying the probabilities down each limb of the tree and summing those with positive outcomes (Martin et al., 2007).

If all surveillance results are negative, the probability of disease freedom can be estimated ie. What is the probability the region is free from CWD, given the surveillance system failed to find the disease, or $\Pr(D-|T-)$? In other words, this is the negative predictive value which can be calculated using Bayes theorem, using the surveillance system sensitivity and a prior probability of disease freedom (Martin et al., 2007). The prior probability is a function of the previous surveillance evidence accumulated over time and the ongoing risk of introduction.

SCOPE OF THE MODEL

The sensitivity of Saskatchewan's CWD surveillance system in free-ranging mule and white-tailed deer was calculated using methods previously described by Martin et al. (2007). A scenario tree model was developed to depict the surveillance system. In order to estimate disease freedom, surveillance results from the south eastern part of the province were analyzed because CWD has not been found in free-ranging populations. For this analysis, actual animal surveillance results from 1997 to 2006 were used from Wildlife Management Zones (WMZs) 1, 15-18, 20, 21, 31-42, 48, 49, 56-59 (Figure 2).

CWD SURVEILLANCE SYSTEM COMPONENTS

The surveillance system (SS) for CWD in Saskatchewan has two surveillance system components (SSc); a surveillance component consisting of submissions of clinically ill animals (diseased, dying, dead) (SSc_3D) and a surveillance component consisting of submissions from hunter-killed animals (SSc_hunter). Samples from both components are submitted along with information on species, age, sex, and geographic location. Location information includes point locations when available (latitude, longitude); surveillance results are further aggregated into wildlife management zones (WMZ). All samples were tested via immunohistochemistry (Spraker et al., 2002). All results in these WMZs from 1997-2006 were negative.

1) Clinically ill animals – Diseased, dying, dead submissions (SSc_3D)

Samples from clinically ill or dead animals were collected and submitted for pathologic diagnosis primarily through conservation officers. Concerned citizens could call their local Saskatchewan Ministry of the Environment office to report a sick animal. A conservation officer would then attend the site, euthanize the animal (if required) and submit the carcass (or portions thereof) to the Canadian Cooperative Wildlife Health Center (CCWHC) for necropsy. Samples were submitted primarily to determine cause of death, for food safety disposition, or for forensic investigations. For the purposes of this analysis,

animals from the SSc_3D were further categorized by assessed CWD risk: 1) *CWD Suspect*- found alive in poor or very poor body condition exhibiting neurologic symptoms such as salivation, depression, increased or decreased flight zone, low head carriage, hyper-excitability, teeth grinding or unable to rise, 2) *Elevated CWD Risk*- found alive in poor body condition, and not exhibiting neurological symptoms or dead in poor or very poor body condition and 3) *Low CWD Risk*- found dead or alive (and euthanized) in fair, good or very good body condition. Any case missing information on body condition was placed into the *Low CWD risk* category for the purposes of this analysis. From 1997-2006, a total of 120 samples were tested in these 24 WMZs. The distribution of these samples by WMZ is given in Figure 3 and the yearly number of samples is given in Table 1.

2) Hunter-harvested submissions (SSc_hunter)

Hunter-harvested surveillance was initiated in 1997, following the discovery of CWD in a farmed elk herd. From 1997-2006, a total of 2112 samples have been tested from these 25 WMZs. The distribution of these samples by WMZ is given in Figure 4. Hunter-harvested samples submitted for CWD testing were primarily adult deer because they have a higher probability of being infected. The number of samples submitted each year is given in Table 2. Heads (including brain, retropharyngeal lymph nodes, and tonsils) from hunter-harvested animals were submitted via hunters to the local Ministry of the Environment office. Samples were then sent to the CCWHC which conducted the testing (with Prairie Diagnostic Laboratories) and reported results. For the purposes of this analysis, all hunter-harvested animals are assumed to be healthy and in good body condition because if after an animal is shot, it is found to be in poor body condition or have significant disease, it will be submitted through the SSc_3D. Therefore, all animals submitted through the hunter-harvest program are considered '*Low CWD Risk*' based on the risk categories defined previously.

DATA SOURCES

1) Canadian Cooperative Wildlife Health Centre (CCWHC)

Records of all wild white-tailed deer and mule deer samples submitted to the CCWHC were examined. This included diseased animals submitted by conservation officers, and those submitted through the hunter-harvested CWD program. Information obtained from these records included species, history, age, sex, location, body condition, necropsy and histology results.

2) Saskatchewan Ministry of the Environment

The Saskatchewan Ministry of the Environment provided information on population estimates and geographic distribution of both mule and white-tailed deer.

3) CWD Literature

Published literature was used to model the sensitivity of immunohistochemistry and relative risk differences of target testing.

4) Expert opinion/Author

Expert opinion or the opinion of the author was used when data were not available for the model.

SCENARIO TREE

A simplified scenario tree was developed to model the surveillance system using the technique described by Martin et al. (2007) and the sensitivity of the surveillance system at a pre-set prevalence (design prevalence) was calculated. Surveillance results from white-tailed deer and mule deer were analyzed together. Groups with equivalent risk of disease represent individual limbs in the tree. For example, some groups, such as clinical suspects greater than 1 year, have a higher probability of being diseased, when compared to normal, healthy animals greater than 1 year. The nodes of the scenario tree are described in Table 3 and the scenario tree describing the surveillance system is given in

Figure 5. Each node is conditional on all higher nodes. The unit of analysis was the individual animal.

MODEL INPUTS

Risk parameters for branch probabilities were estimated and modeled using distributions (Pert and Uniform) in order to account for uncertainty using stochastic modeling. Model inputs are described in more detail below and summarized in Table 4.

Region

Region was considered a risk factor for this analysis. CWD is transmitted horizontally from an infected animal (Miller and Williams, 2003) or through exposure to infected environments (Miller et al., 2004). It follows that free-ranging animals in close proximity to areas where CWD is known to exist (in the wild or on game farms) are at higher risk of infection. The increase in surveillance sensitivity gained by targeting higher risk areas is a function of 1) the proportion sampled from those regions and 2) the relative risk difference of infection between areas of higher and lower risk (Martin et al., 2007). For this analysis, WMZs were divided into either the *HighRisk* branch or the *Other*. Only proximity to known infected wild cervids was considered, therefore WMZs 42, 49, 58, and 59 were considered to be at higher risk because these zones were adjacent to known positive WMZs. The relative risk (RR) of a *HighRisk* WMZ being infected relative to *Other* was modeled conservatively via a Pert distribution [Pert (1, 2, 3)]. The RR was then adjusted by the underlying estimated proportion of deer in the *HighRisk* and *Other* regions to ensure that the weighted average adjusted risk across the limb was 1. The proportion of the population of deer in those WMZs was determined by dividing the number of estimated deer coming from WMZs 42, 29, 58 and 59 by the total number of estimated deer from all WMZs in the study area (Arsenault, 2005).

WMZ status

Within the study region, a WMZ would either be *infected* or *uninfected*. The WMZ design prevalence or 'threshold prevalence' (P^*_H) defines the prevalence assumptions for which the sensitivity is valid and are not subject to uncertainty (Martin et al., 2007). For this study, sensitivity was calculated under two P^*_H scenarios: 0.04 and 0.08. This is equivalent to 1 or 2 WMZs infected of the 25 WMZs, respectively.

Age

Young animals have a much lower probability of disease, and therefore testing of young animals is of less value than testing animals greater than 1.5 years of age. For this analysis, samples were divided into two categories: <1 year (*Juvenile*) and = 1 year (*Adult*), based on the quality of age information available. The risk of *Adult* being infected relative to *Juvenile* was modeled conservatively via a pert distribution [Pert (10,15,20)]. The underlying population structure was estimated using SMOE's Cooperative Deer Management Survey (CDMS) (Arsenault, 2005).

Health status

Animals were divided into three risk categories: 1) A *CWD Suspect* was defined as any animal observed to have been in poor or very poor body condition with accompanying neurologic signs (salivation, depression, increased or decreased flight zone, low head carriage, teeth grinding, unable to rise) potentially consistent with CWD; 2) An animal with *Elevated CWD Risk* was any other animal observed to be in poor or very poor body condition. Animals with clinical disease are assumed to be at higher risk for death due to motor vehicle collisions, gunshot wounds, predators, aspiration pneumonia and sudden death; 3) An animal with *Low CWD Risk* was any animal found dead and observed to be in fair, good or very good body condition. *CWD suspects* and those with *Elevated CWD Risk* have a higher risk of infection relative to those with *Low CWD Risk*. The risk of *CWD suspects* and *Elevated CWD Risk* was modeled using uniform

distributions (13.2, 25) and (3, 13) relative to *Low CWD Risk*. These estimates were derived from the literature (Miller et al., 2000; Krumm et al., 2005) and using actual Saskatchewan surveillance results in areas where CWD has been identified. The proportions of animals which die each year that are *CWD Suspects*, *Elevated CWD Risk* and *Low CWD Risk* were estimated using historical unpublished data from the Saskatchewan wildlife passive surveillance system.

Animal status

The threshold prevalence or design prevalence at the individual animal level (P^*_U) is defined as the probability that an individual animal is infected, given that the herd is infected. For this analysis, values of 0.01, 0.02, and 0.05 (1%, 2% and 5% prevalence) were used to demonstrate the fluctuation in sensitivity and disease freedom results depending on surveillance goals.

Test positive

The probability of a sample testing positive is dependant on the sensitivity of the diagnostic test, in this case immunohistochemistry (IHC). The specificity of this test was assumed to be 100%, as all positive samples are sent for confirmatory testing. For this analysis, the sensitivity was modeled conservatively using a Pert distribution (0.906, 0.973, 0.997) (Miller et al., 2002).

Confirmed positive

This input describes the probability that a test positive sample will be confirmed positive. For the purposes of this analysis and to maintain simplicity of the model, it was assumed that all samples that were initially test positive were confirmed positive with a perfect test (Se 100%, Sp 100%).

SSC SENSITIVITY CALCULATION

Surveillance results were analyzed yearly rather than monthly because CWD is a slow spreading disease with a relatively long duration of clinical disease. The sensitivity of the surveillance system was calculated for each WMZ using the binomial probability formula (Martin et al., 2007, Eq. 8). The 'WMZ sensitivity' is the probability that at least one animal will test positive after testing n animals if the WMZ is infected at prevalence P^*_U (Martin et al., 2007). The overall surveillance system sensitivity was calculated using SSc_{3D} and SSc_{hunter} for each year from 1997-2006. The overall sensitivity is the probability that at least one WMZ will test positive when infection is present at a pre-determined design prevalence or $\Pr(T^+ | D^+)$.

STOCHASTIC SIMULATION

Monte Carlo stochastic simulation of results was conducted using PopTools 2.7.5. (Hood, 2006). A fixed random number seed of 1 was used with 3000 iterations. Uncertainty around region, age, health status and IHC sensitivity was modeled using the distributions previously described.

PROBABILITY OF CWD FREEDOM

The probability of the region being free of disease given our surveillance results, or $\Pr(D^- | T^-)$, was modeled each year using Bayes theorem. The posterior probability of disease freedom is a function of the prior probability of disease freedom and the sensitivity of the surveillance system. The prior probability of being free was set at 0.676, based on the prevalence among the other 37 WMZs in the western part of the province (12/37 or 32.4%). Because the risk of introduction is ongoing and needed to be accounted for, the posterior probability of disease from the previous year was discounted by the probability of introduction to obtain the new prior probability of disease (Martin et al. 2007, Eq. 18). There was no information available to model the probability of introduction, therefore low risk (0.01) and high risk scenarios (0.1) were used.

SENSITIVITY RATIO

A sensitivity ratio was calculated to compare the sensitivity of *SSc_3D* with *SSc_hunter* in order to help describe the benefit of targeted testing of high risk animals. The sensitivity of each component was simulated using hypothetical data and the components were compared. Hypothetical data consisted of 3 animals tested per WMZ (n=75) in each of the *SSc_hunter* and *SSc_3D* (*CWD suspect*).

5.4 RESULTS

SURVEILLANCE SENSITIVITY CALCULATION

The overall mean sensitivity of both CWD surveillance components (*SSc_hunter* with *SSc_3D*) combined from 1997 to 2006 in the CWD-negative area under the six design prevalence scenarios (previously described) is presented in Figure 6 and Table 5. Surveillance sensitivity was poor from 1997 to 1999 and increased dramatically from 2000 to 2003, reflecting increased intensity of hunter-harvested surveillance during that time. Although the largest number of clinical samples in one year was submitted in 1997 (n=40), the sensitivity remained low given the nature of the samples; greater than half were in young animals (n=21), and the majority of adult animals were in good body condition (n=13). Sensitivity results were largely dependent on the design prevalence, with as much as a mean difference of 56.7% in the peak year, 2001 between the most conservative Scenario 1 (1/25 WMZs infected at 1% prevalence) and the least conservative Scenario 6 (2/25 WMZs infected at 5% prevalence).

PROBABILITY OF DISEASE FREEDOM

The probability of the eastern part of the province being free of disease given our surveillance results, or $\Pr(D^- | T^-)$, is given in Figure 7 and Table 6. Scenario 1 (1/25 WMZs infected at 1% prevalence) and Scenario 6 (2/25 WMZs infected at 5% prevalence) were modeled using low and high risk probability of introduction scenarios (0.01 and 0.1, respectively). Results clearly demonstrate the need for

increased vigilance and surveillance intensity as the risk of introduction increases. Alternatively, if the risk of disease introduction is low, confidence of disease freedom increases over time as surveillance results accumulate. Based on surveillance findings, if the risk of introduction from 1997-2006 was very low (0.01; ie. disease is introduced once in 100 years), the probability that CWD was present in at least 2 WMZs at 5% prevalence or greater is negligible.

SENSITIVITY RATIO

The sensitivity ratio is a measure of the performance of the *SSc_3D* relative to *SSc_hunter*. Results from the hypothetical data comparing the two components *SSc_hunter* with *SSc_3D* under the six different scenarios are presented in Table 7. Comparing the relative value of testing 75 CWD suspects to 75 hunter-harvested animals, we see that as the prevalence increases, the relative value of testing clinical animals' decreases.

5.5 DISCUSSION

The intent of this analysis was not to make definitive claims regarding the status of CWD in wild deer in eastern Saskatchewan, but rather to demonstrate the potential of using this quantitative methodology in order to calculate CWD surveillance system sensitivity, probability of disease freedom and to quantify the gain in sensitivity using targeted surveillance in wild deer.

It is clear from this analysis that the overall sensitivity of a surveillance system is highly dependent on what the surveillance goals are; the lower the prevalence you wish to detect, the higher the surveillance intensity required. Although this typically means an increased number of samples are required, this can be augmented with targeted strategies, such as was demonstrated by targeting CWD suspects. Although the overall number of CWD suspects that were examined using the 3D surveillance system was low during this time period, the sensitivity ratio demonstrates that testing more of these suspects would help

boost sensitivity dramatically in areas of low prevalence and ultimately improve confidence of disease status.

Additionally, this analysis demonstrates the susceptibility of disease freedom confidence in situations where there is an ongoing high risk of disease introduction. Risk of disease introduction can increase slowly over time from migration of animals from positive CWD areas or increase dramatically via intentional movement of infected animals into disease free areas. For this reason, ongoing risk must be continually assessed and used to adjust surveillance system goals. Close collaboration and cooperation between provincial agricultural (game–farming industry) and wildlife organizations is essential.

One of the main challenges in using quantitative methodologies is that they often work best in situations where ample data exist to obtain good estimates for model inputs. This presents a significant challenge in wildlife disease scenarios for two reasons; 1) wildlife populations are inherently more diverse and difficult to study than domestic populations due to their elusiveness and large geographic distribution making it more difficult to acquire accurate estimates for modeling; and 2) wildlife management and research receives less funding overall than research used to support agricultural industry and trade. Ultimately, the usefulness of these methods in wildlife situations will depend on obtaining an overall better understanding of the distribution and dynamics of diseases in wildlife populations. In this case, the model would benefit from more accurate population estimates and better estimates of the risk of CWD introduction in different scenarios (natural movement or migration of infected animals or spillover from positive game farms). Finally, identifying and incorporating other herd and animal level risk factors for CWD (eg. sex, density, artificial baiting, etc.) would help to further refine the model.

This quantitative methodology would be useful in developing surveillance strategies for disease detection at the regional level. In each 'negative region', a cost-benefit analysis could be conducted to determine the most economic way to meet surveillance objectives. That is, would it be more beneficial to increase the number of harvested samples through increased tags, incentives, etc. as compared to running campaigns asking people to report injured animals, and picking up road kill? Obviously, in areas where the human population density is low, public relations campaigns and relying on 3D passive surveillance systems to achieve surveillance goals may be unrealistic. On the other hand, in highly populated areas, surveillance goals may be easily met through improving the number of 3D passive surveillance submissions. A point system, similar to that developed by the OIE for BSE surveillance (http://www.oie.int/eng/normes/mcode/en_chapitre_1.11.6.htm), could be developed, making it more user-friendly and comprehensible for conservation officers and managers to meet local surveillance system goals.

There are many limitations worth mentioning. Ultimately, the model could have been made more complex and more risk nodes could have been included in the model. However because the intent of this analysis was to demonstrate the potential of this methodology under different scenarios, and data were sparse to non-existent in many situations, simplicity was maintained at the expense of limiting the model. A significant limitation of this model is the lack accurate population and local mortality estimates, and incomplete information on the risk differences between high risk and low risk regions. The use of wildlife management zones (WMZs) as a surrogate measure for a herd or population unit also limits the use of this model, however there were no other alternatives to represent biological clustering of CWD. Additionally, deer population density of the different regions was not included in the model even though it may also be considered a risk factor for disease spread. Density was excluded because data were not available for the differential risk difference between high density and low density situations. Both white-tailed deer and mule deer samples were treated

the same, even though there are many differences between the two species (eg. they occupy different habitats, are at different densities in different regions, and would have different contact rates within and between species). Although elk are susceptible to CWD, they were not included in this model to preserve simplicity. Finally, the risk from game farm animals was not incorporated into the model, as data were not available.

Although the model was simplified and didn't include different risk nodes like sex, and density, including these risk factors would likely not have affected the model outputs substantially, especially with the SSc_3D surveillance component, due to the overall low number of submissions to the surveillance system. These factors would be more important as the number of submissions increases and should be considered for future models.

5.6 CONCLUSION

The methodology, as outlined by Martin et al. (2007), has been shown to be useful for estimating surveillance sensitivity, probability of disease freedom and to demonstrate the benefit of risk-based target testing. Further, it can and should be used as part of the surveillance evaluation process in order to measure if surveillance systems are meeting surveillance objectives and to demonstrate where surveillance is adequate and where it is not. Ample, good quality data are required to improve the application and robustness of these models in wildlife scenarios.

Based on this analysis, surveillance in the eastern part of Saskatchewan is not adequate to detect CWD at 0.5-1% prevalence. Having said that, this area has not been a focus of surveillance and even with the limited surveillance that has been done, it can be concluded that if the risk of disease introduction was very low, the prevalence is not 5% or higher. The use of more targeted surveillance strategies should be further explored to help better meet surveillance objectives.

Figure 1. Locations where chronic wasting disease has been detected in wild deer in Saskatchewan; 2000-2008

Source: http://wildlife1.usask.ca/en/cwd/chronic_wasting_disease.php

The Triangles Represent Locations of CWD Positive Wild Cervids in Saskatchewan, 2000-2008.
Numbered Areas are Wildlife Management Zones.

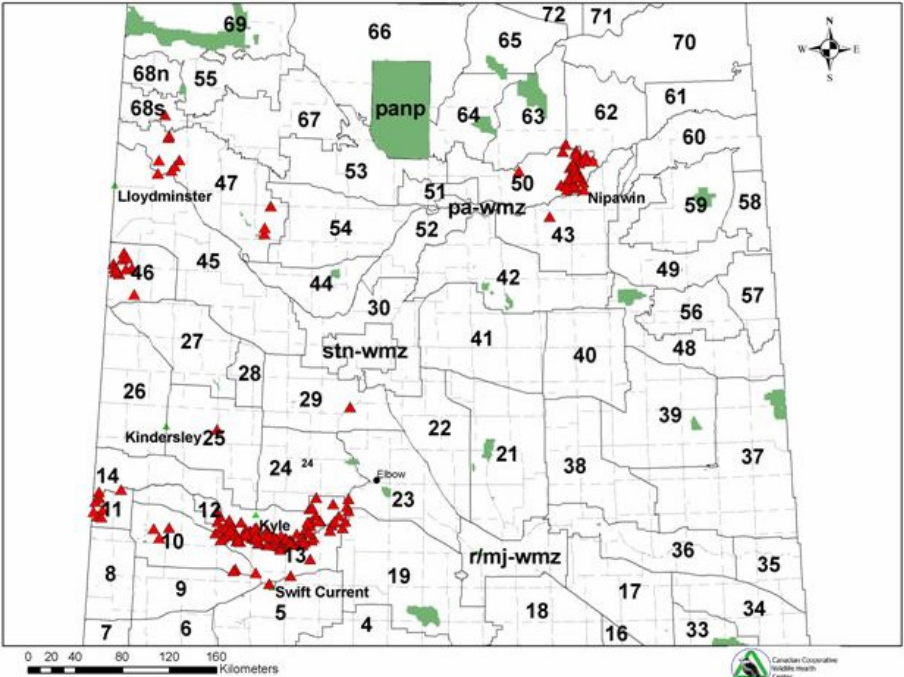


Figure 2 - Map of area showing the eastern Saskatchewan WMZ that were included in this analysis (1, 15-18, 20, 21, 31-42, 48, 49, 56-59)

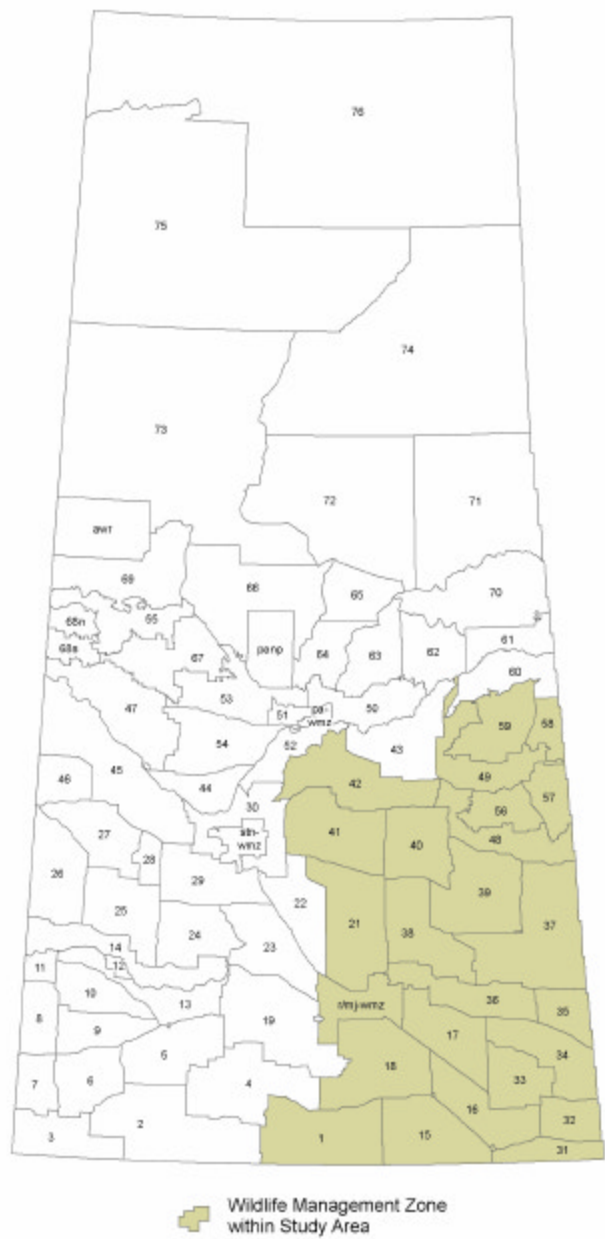
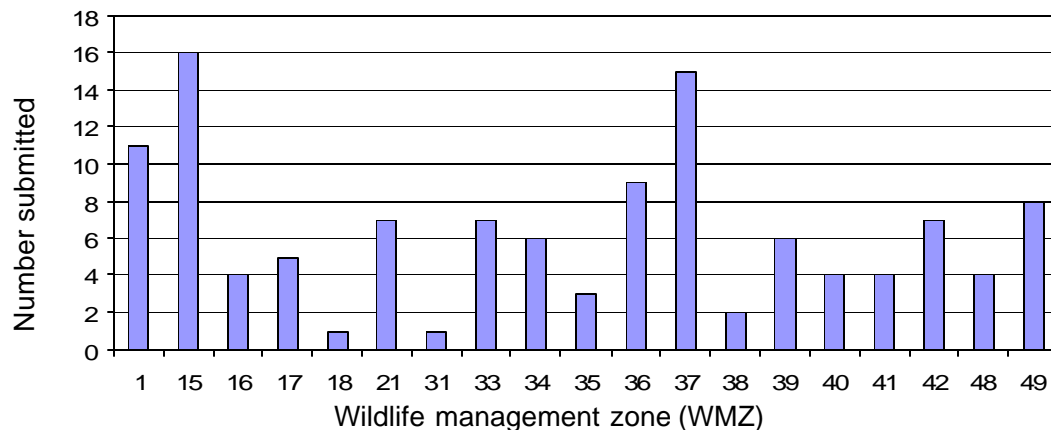


Figure 3 – Number of 3D clinical samples submitted from wild mule and white-tailed deer in eastern WMZs (1, 15-18, 20, 21, 31-42, 48, 49, 56-59)*; 1997-2006



* n=0 samples submitted in zones 20, 32, 56, 57, 58, 59

Table 1 – Yearly number of clinical samples submitted from wild mule and white-tailed deer in eastern WMZs (1997-2006)

Year	Number of samples submitted
1997	40
1998	3
1999	5
2000	6
2001	25
2002	7
2003	5
2004	11
2005	11
2006	7
TOTAL	120

Figure 4 – Number of hunter-harvested samples (mule and white-tailed deer) submitted from eastern WMZs (1, 15-18, 20, 21, 31-42, 48, 49, 56-59) from 1997-2006

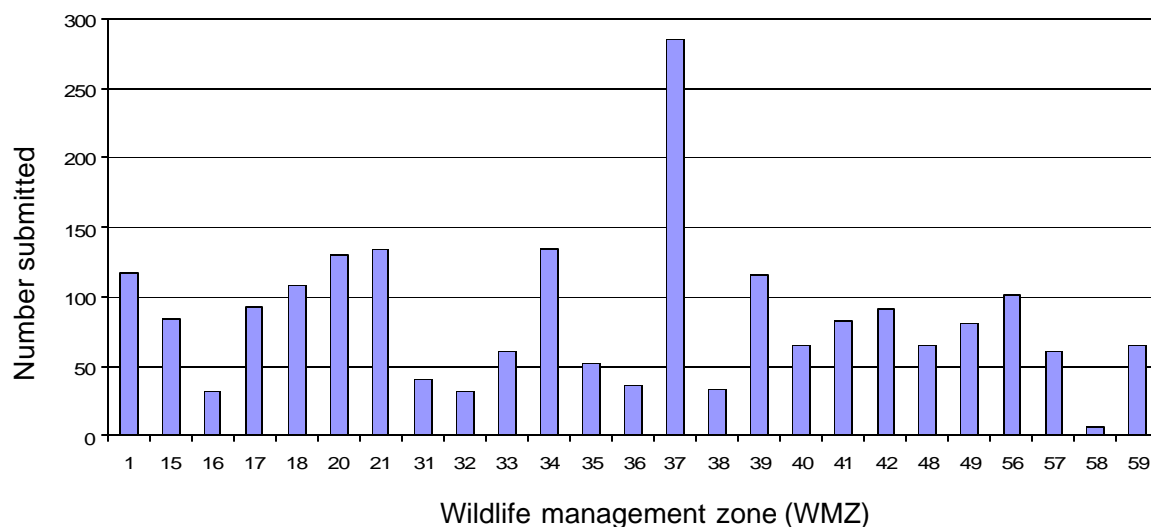


Table 2 – Yearly number of hunter-harvested samples from wild mule and white-tailed deer in eastern WMZs, submitted for testing through the *SSc_Hunter* surveillance component from 1997-2006

Year	Number of samples
1997	16
1998	7
1999	22
2000	182
2001	570
2002	643
2003	493
2004	5
2005	83
2006	91
TOTAL	2112

Table 3 - Infection, detection and risk category nodes in the scenario tree modeling CWD surveillance in wild mule and white-tailed deer, including potential outcomes at each node

Node	Name	Type of Node	Outcomes	Next Node
1	Region	Risk category	- High risk - Other	2 2
2	WMZ infected	Infection	- Infected - Not infected	3 End
3	Age	Risk category	- Adult (=1 yr) - Juvenile (<1yr)	4 4
4	Health status	Risk category	- CWD suspect ¹ - Elevated CWD risk - Low CWD risk (other)	5 5 5
5	Animal infected	Infection node	- Infected - Not infected	6 End
6	Test positive	Detection	- Yes - No	7 End
7	Confirmed positive	Detection	- Yes - No	End End

¹ Clinical suspect- poor body condition with accompanying neurologic signs such as salivation, depression, increased or decreased flight zone, low head carriage, teeth grinding or unable to rise

Table 4 – Parameter estimates used in the scenario tree model for surveillance of chronic wasting disease in Saskatchewan

Parameter	Point estimate or distribution of value	Source
<i>Region</i> RR in a region adjacent to a CWD positive area (<i>HighRisk</i>) relative to <i>Other</i> Proportion of deer in <i>HighRisk</i> area	Pert (1,2,3) 0.122	Author Arsenault, 2005
<i>P^{*H}</i> 1 of 25 WMZs infected 2 of 25 WMZs infected	0.04 0.08	Author
<i>Age</i> RR <i>Adult</i> : <i>Juvenile</i> Proportion of adults	Pert (10,15,20) 0.618	Author Arsenault, 2005
<i>Health status</i> RR <i>CWD Suspect</i> RR <i>Elevated CWD Risk</i> Proportion <i>CWD Suspect</i> Proportion <i>Elevated Risk</i> Proportion of <i>Low Risk</i>	Uniform (13.2, 25) Uniform (3,13) 0.022 0.353 0.625	Miller et al, 2000; unpbl. SK data, Krumm et al, 2005; Author Unpbl. SK data
<i>P^{*U}</i> 1% prevalence 2% prevalence 5% prevalence	0.01 0.02 0.05	Author
<i>Test positive</i> Pr (T+ D+)	Pert (0.91, 0.97,1)	Miller et al, 2002

The flowchart illustrates the CWD testing algorithm for deer and elk, starting with the **REGION** and branching into **High risk** and **Other** categories.

- High risk:**
 - Probability: $Pr_HighRisk$
 - Notes: Same nodes as for Other
- Other:**
 - Probability: Pr_Other
 - WMZ STATUS:**
 - infected:** Probability P^*_H , leading to a node with the same structure as the 'Other' branch.
 - uninfected:** Probability $1-P^*_H$, leading to a terminal node (triangle).
 - Adult:**
 - Probability: R_Adult
 - Pr_Adult:**
 - Probability: Pr_Adult
 - Other:**
 - Probability: Pr_Other
 - Notes: Same as for Adult
 - Juvenile:**
 - Probability: $R_Juvenile$
 - Notes: Same as for Adult
 - Clin Suspects:**
 - Probability: $R_Clinical$
 - ANIMAL STATUS:**
 - Probability: $Pr_Clinical$
 - Notes: Same as for Emac
 - Elevated CWD risk:**
 - Probability: R_Emac
 - ANIMAL STATUS:**
 - Probability: Pr_Emac
 - infected:** Probability P^*_U , leading to a node with the same structure as the 'Other' branch.
 - uninfected:** Probability $1-P^*_U$, leading to a terminal node (triangle).

The flowchart continues with **CWD TEST** and **CONFIRMATORY CWD TESTING** steps, leading to final outcomes like **C_TESTSe Positive** or **C_TESTSe Negative**.

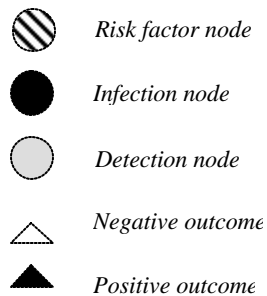
99

Figure 6 - CWD mean surveillance system sensitivity results (1997-2006) incorporating both SSc_3D and SSc_hunter surveillance components.

Scenario 1: Surveillance sensitivity in detecting CWD if one WMZ is infected at 1% prevalence within the region (P^*_H 0.04; P^*_U 0.01); Scenario 2: Surveillance sensitivity in detecting CWD if two WMZs are infected at 1% prevalence within these region (P^*_H 0.08; P^*_U 0.01); Scenario 3: Surveillance sensitivity in detecting CWD if one WMZ is infected at 2% prevalence within the region (P^*_H 0.04; P^*_U 0.02); Scenario 4: Surveillance sensitivity in detecting CWD if two WMZs are infected at 2% prevalence within the region (P^*_H 0.08; P^*_U 0.02); Scenario 5: Surveillance sensitivity in detecting CWD if one WMZ is infected at 5% prevalence within the region (P^*_H 0.04; P^*_U 0.05); Scenario 6: Surveillance sensitivity in detecting CWD if two WMZs are infected at 5% prevalence within the region (P^*_H 0.08; P^*_U 0.05).

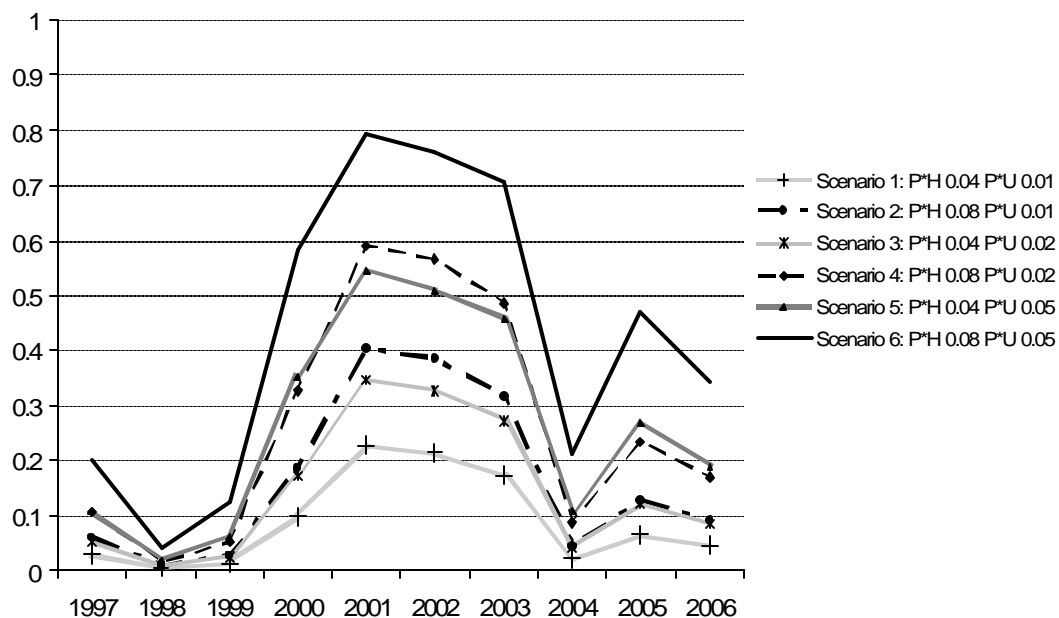


Table 5 - CWD mean (95%CL) surveillance system sensitivity results (1997-2006) incorporating both SSc_3D and SSc_hunter surveillance components

Scenario 1: Surveillance sensitivity in detecting CWD if one WMZ is infected at 1% prevalence within the region (P^*_H 0.04; P^*_U 0.01); Scenario 2: Surveillance sensitivity in detecting CWD if two WMZs are infected at 1% prevalence within the region (P^*_H 0.08; P^*_U 0.01); Scenario 3: Surveillance sensitivity in detecting CWD if one WMZ is infected at 2% prevalence within the region (P^*_H 0.04; P^*_U 0.02); Scenario 4: Surveillance sensitivity in detecting CWD if two WMZs are infected at 2% prevalence within the region (P^*_H 0.08; P^*_U 0.02); Scenario 5: Surveillance sensitivity in detecting CWD if one WMZ is infected at 5% prevalence within the region (P^*_H 0.04; P^*_U 0.05); Scenario 6: Surveillance sensitivity in detecting CWD if two WMZs are infected at 5% prevalence within the region (P^*_H 0.08; P^*_U 0.05).

	Scenario 1: P^*_H 0.04; P^*_U 0.01	Scenario 2: P^*_H 0.08; P^*_U 0.01	Scenario 3: P^*_H 0.04; P^*_U 0.02	Scenario 4: P^*_H 0.08; P^*_U 0.02	Scenario 5: P^*_H 0.04; P^*_U 0.05	Scenario 5: P^*_H 0.08; P^*_U 0.05
Year	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)
1997	0.030 (0.021;0.041)	0.060 (0.041;0.081)	0.052 (0.038;0.067)	0.106 (0.078;0.136)	0.107 (0.087;0.129)	0.204 (0.167;0.244)
1998	0.004 (0.003;0.005)	0.009 (0.007;0.010)	0.008 (0.006;0.010)	0.017 (0.013;0.021)	0.020 (0.016;0.025)	0.040 (0.032;0.49)
1999	0.013 (0.011;0.017)	0.027 (0.021;0.034)	0.026 (0.020;0.032)	0.053 (0.042;0.066)	0.064 (0.052;0.081)	0.124 (0.101;0.155)
2000	0.098 (0.076;0.119)	0.186 (0.148;0.225)	0.172 (0.137;0.207)	0.326 (0.266;0.385)	0.353 (0.295;0.407)	0.583 (0.507;0.654)
2001	0.227 (0.190;0.264)	0.404 (0.345;0.462)	0.347 (0.301;0.392)	0.591 (0.530;0.650)	0.543 (0.503;0.585)	0.794 (0.758;0.835)
2002	0.215 (0.179;0.250)	0.385 (0.327;0.440)	0.328 (0.284;0.369)	0.565 (0.504;0.621)	0.508 (0.470;0.544)	0.760 (0.725;0.797)
2003	0.173 (0.142;0.202)	0.316 (0.265;0.366)	0.273 (0.233;0.311)	0.487 (0.426;0.544)	0.456 (0.414;0.496)	0.707 (0.661;0.752)
2004	0.022 (0.014;0.032)	0.044 (0.028;0.063)	0.042 (0.027;0.060)	0.087 (0.055;0.124)	0.108 (0.069;0.154)	0.210 (0.135;0.300)
2005	0.065 (0.051;0.083)	0.127 (0.099;0.159)	0.120 (0.094;0.150)	0.234 (0.186;0.289)	0.269 (0.217;0.330)	0.469 (0.390;0.558)
2006	0.047 (0.036;0.057)	0.092 (0.072;0.112)	0.085 (0.067;0.103)	0.169 (0.135;0.204)	0.189 (0.154;0.223)	0.343 (0.285;0.399)

Figure 7 – Mean probability of freedom of CWD from 1997-2006 in Wildlife Management Zones (WMZs) 1, 15-18, 20, 21, 31-42, 48, 49, 56-59, estimated from surveillance (SSc_3D and SSc_hunter)

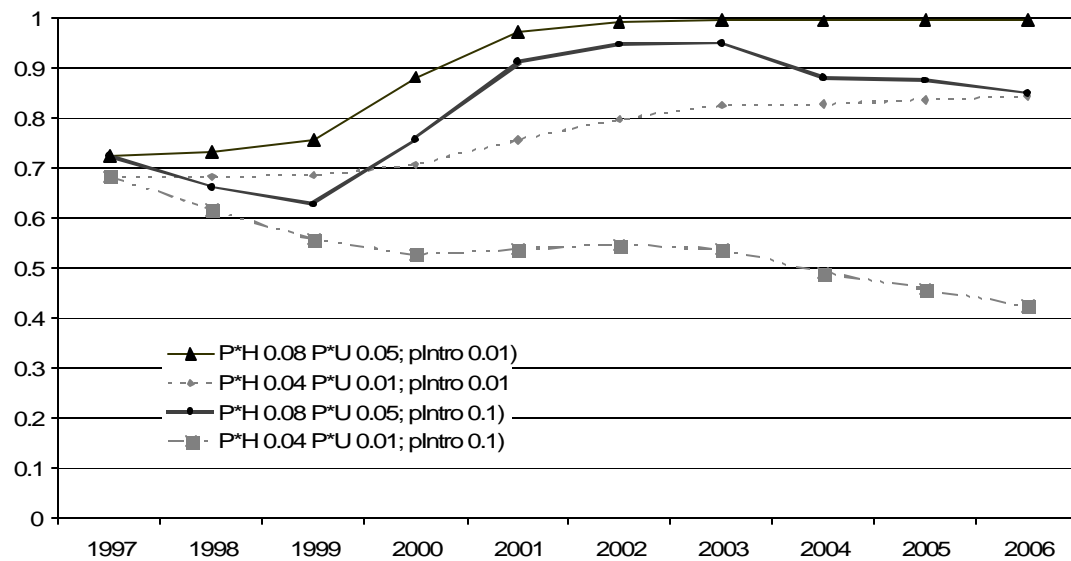


Table 6 – Mean (95%CL) probability of CWD freedom 1997-2006 in Wildlife Management Zones (WMZs) 1, 15-18, 20, 21, 31-42, 48, 49, 56-59, estimated from surveillance (SSc_3D and SSc_hunter)

	P*_H 0.04; P*_U 0.01 plntro 0.1	P*_H 0.04; P*_U 0.01 plntro 0.01	P*_H 0.08; P*_U 0.05 plntro 0.1	P*_H 0.08; P*_U 0.05 plntro 0.01
Year	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)	Mean (95% CL)
1997	0.682 (0.680-0.685)	0.682 (0.680-0.685)	0.724 (0.714-0.733)	0.724 (0.714-0.734)
1998	0.615 (0.613-0.617)	0.683 (0.680-0.685)	0.661 (0.652-0.671)	0.731 (0.722-0.742)
1999	0.557 (0.555-0.560)	0.685 (0.682-0.688)	0.626 (0.612-0.643)	0.756 (0.742-0.772)
2000	0.527 (0.521-0.535)	0.706 (0.700-0.713)	0.757 (0.719-0.796)	0.881 (0.878-0.905)
2001	0.539 (0.521-0.558)	0.756 (0.742-0.771)	0.911 (0.883-0.938)	0.971 (0.961-0.983)
2002	0.545 (0.518-0.572)	0.797 (0.777-0.816)	0.947 (0.934-0.964)	0.990 (0.989-0.996)
2003	0.538 (0.505-0.571)	0.825 (0.802-0.847)	0.948 (0.940-0.963)	0.995 (0.996-0.999)
2004	0.490 (0.459-0.521)	0.827 (0.804-0.850)	0.880 (0.866-0.902)	0.994 (0.996-0.998)
2005	0.458 (0.426-0.490)	0.836 (0.812-0.860)	0.876 (0.854-0.907)	0.995 (0.997-0.999)
2006	0.424 (0.392-0.456)	0.841 (0.816-0.866)	0.848 (0.824-0.879)	0.995 (0.997-0.999)

Table 7 - Mean (minimum; maximum) of SSc_3D and SSc_hunter and sensitivity ratio using 6 different scenarios. Scenario 1: Surveillance sensitivity in detecting CWD if one WMZ is infected at 1% prevalence within the zone; Scenario 2: Surveillance sensitivity in detecting CWD if two WMZs are infected at 1% prevalence within these zones; Scenario 3: Surveillance sensitivity in detecting CWD if one WMZ is infected at 2% prevalence within the zone; Scenario 4: Surveillance sensitivity in detecting CWD if two WMZs are infected at 2% prevalence within these zones; Scenario 5: Surveillance sensitivity in detecting CWD if one WMZ is infected at 5% prevalence within the zone; Scenario 6: Surveillance sensitivity in detecting CWD if two WMZs are infected at 5% prevalence within these zones;

Scenario	Design prevalence	SSc_3D	SSc_hunter	Sensitivity ratio
1	P ^{*H} .04*P ^{*U} .01	0.441 (0.347; 0.535)	0.035 (0.028; 0.043)	12.537
2	P ^{*H} .08*P ^{*U} .01	0.664 (0.547; 0.770)	0.069 (0.054; 0.084)	9.606
3	P ^{*H} .04*P ^{*U} .02	0.572 (0.491; 0.637)	0.068 (0.054; 0.083)	8.381
4	P ^{*H} .08*P ^{*U} .02	0.818 (0.747; 0.874)	0.132 (0.105; 0.159)	6.199
5	P ^{*H} .04*P ^{*U} .05	0.657 (0.647; 0.727)	0.157 (0.127; 0.187)	4.183
6	P ^{*H} .08*P ^{*U} .05	0.883 (0.881; 0.931)	0.290 (0.238; 0.340)	3.048

6. CONCLUSION

With demands for wildlife surveillance information increasing and diversifying, laboratory submission-based passive surveillance systems can play an increasingly important role in disease discovery and monitoring. Although this is an overall biased survey with targeting of the high risk “3D” (diseased, dead, dying) subset of the population, it can be an effective approach to detecting new diseases at low prevalence, as the likelihood of finding a disease in this population is increased. The term ‘passive’ is misleading, in that it implies that little work is required to maintain such a surveillance system. In fact the opposite is true; maintaining financial support and vigilance can be very difficult and expensive. In Saskatchewan, spatial, temporal and demographic submission patterns were variable and heavily dependent on specific, intermittent research projects. Although these projects were very valuable in providing resources that otherwise would not have been available for wildlife disease surveillance, they ultimately affected the surveillance outcomes. If 3D surveillance is to be used as an effective surveillance system for wildlife diseases, work to improve this system should include improving submission rates across all regions and acquiring better quality information. It follows that more consistent funding of surveillance of wildlife populations would dramatically improve this system.

Surveillance for chronic wasting disease in wild deer in Saskatchewan has proved to be very challenging due to factors relating to the disease itself but also due to a poor understanding of population structures, the lack of accurate denominator data and to limited resources, a challenge faced by most wildlife authorities. With these challenges in mind, regular, systematic surveillance evaluations are essential to ensure that surveillance objectives are being met efficiently and effectively. This can be achieved through either qualitative or quantitative reviews. Qualitative reviews may be more appropriate in many situations where there are fewer data, as much insight can be gained simply through the systematic review process of clearly stating objectives, defining

priorities, and measuring whether objectives are being met. The recent development of methods using quantitative analysis, as was demonstrated in this manuscript, is an exciting new option for evaluating surveillance systems. Although these methodologies were developed primarily for providing evidence of disease freedom in trade of domestic animals and require abundant, good quality data, this methodology can be very valuable in wildlife situations provided adequate data is available. This methodology is also valuable in estimating surveillance system sensitivity and quantifying the gain in sensitivity achieved through targeted testing. Using this methodology to model different surveillance scenarios also helped to clearly demonstrate where more data were required for more robust modeling.

The establishment of the hunter-harvest surveillance program has been critical in understanding the incursion of CWD in wild populations in Saskatchewan. It has helped to obtain estimates of prevalence in regions where CWD has been detected and to monitor the impact of disease control efforts. Large numbers of samples have been tested in CWD-positive areas as part of the population reduction initiatives. The trade-off has been that in many areas with current 'negative' status, few samples have been submitted, providing little evidence that the disease is not present. Further evaluation of the level of surveillance required in CWD-positive areas should occur to prevent oversampling in these areas, and to save more resources for disease detection in 'CWD-negative' areas.

In theory, hunter-harvest surveillance is less efficient at detecting disease in areas of low prevalence than targeting '3D' animals. Clinically ill animals, especially CWD suspects, have a much higher probability of being infected. Although this is true in theory, there are many challenges that impair the sensitivity of '3D' surveillance that must be overcome for it to be effective. The current clinical surveillance component is not sufficient to fill in the 'gaps' and the primary reason for poor performance by this component has been a very low submission rate. Enhancing this system in areas where the disease has *not* been detected would improve sensitivity. This will likely require additional resources for

education and awareness campaigns and for front-line staff, but given the importance of managing this disease, would be money well spent. The overall sensitivity of a surveillance system and confidence of disease freedom are highly dependent on detection prevalence and the ongoing risk of disease introduction. The lower the prevalence, the higher the surveillance intensity required. Additionally, ongoing risk or disease introduction must be continually assessed in order to adjust the value of previous testing. With these two factors in mind, a detection goal of 0.5-1% prevalence is an ambitious surveillance goal, especially in areas where the risk of disease introduction is high. Further refinement of CWD surveillance at the regional level, based on the surveillance goals (estimating prevalence and monitoring trends vs. early disease detection) would help to meet overall surveillance objectives. A point system, similar to that developed by the OIE for BSE surveillance, could be developed, making it more user-friendly and comprehensible for conservation officers and managers to meet local surveillance system goals.

Based on this analysis, surveillance in the eastern part of Saskatchewan is not adequate to detect CWD at 0.5-1% prevalence. Having said that, this area has not been a focus of surveillance and even with the limited surveillance that has been done, it can be concluded that if the risk of disease introduction was very low, the prevalence is not 5% or higher. The use of more targeted surveillance strategies should be further explored to help better meet surveillance objectives.

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